FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS

EVALUATION AND RESEARCH

VACCINES AND RELATED

BIOLOGICAL PRODUCTS ADVISORY COMMITTEE

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Proceedings By:

CASET Associates, Ltd.
Fairfax, Virginia 22030
caset@caset.net

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P R O C E E D I N G S (8:04 a.m.)

Agenda Item: Call to Order and Opening Remarks

DR. STAPLETON: Good morning. I am Jack Stapleton from the University of Iowa, Infectious Diseases, and I am the Chair of today's meeting. I would like to call the meeting to order and ask Christine Walsh to make some announcements, please.

MS. WALSH: Good morning. I am Christine Walsh, the designated federal official for today's meeting of the Vaccines and Related Biological Products Advisory Committee. I would like to welcome all of you to this meeting.

Today's session will consist of presentations that are open to the public as described in the *Federal Register* notice of April 8, 2010. I would like to request that any media inquiries be directed to Miss Shelley Burgess from the Office of Public Affairs, FDA.

I would also like to request that everyone please check your cell phones and pagers and make sure they are off or in the silent mode.

Agenda Item: Conflict of Interest Statement

I would now like to read into public record the conflict of interest statement for today's meeting.

The Food and Drug Administration, FDA, has convened the May 7, 2010 meeting of the Vaccines and Related Biological Products Advisory Committee under the authority of

the Federal Advisory Committee Act, FACA, of 1972. With the exception of the industry representative, all participants of the committee are special government employees, SGEs, or regular federal employees from other agencies, and are subject to the federal conflict of interest laws and regulations.

The following information on the status of this advisory committee's compliance with federal ethics and conflict of interest laws, including but not limited to 18 USC 208 and 712 of the Federal Food Drug and Cosmetic Act, are being provided to participants at this meeting and to the public.

FDA has determined that all members of this advisory committee are in compliance with federal ethics and conflict of interest laws. Under 18 USC 208, Congress has authorized FDA to grant waivers to special government employees and regular government employees who have financial conflicts when it is determined that the agency's need for a particular individual's service outweighs his or her potential financial conflict of interest.

Under 712 of the Food Drug and Cosmetic Act, Congress has authorized FDA to grant waivers to special government employees and regular government employees with potential financial conflicts when necessary to afford the committee their essential expertise. Related to the

discussion of this meeting, members and consultants of this committee have been screened for potential financial conflicts of interest of their own, as well as imputed to them, including those of their spouses or minor children, and for the purpose of 18 USC 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, CREDAs, teaching, speaking, writing, patents and royalties and also primary employment.

For Topic 1, the committee will review and discuss available data regarding the unexpected finding of DNA origination from porcine circovirus, type 1, PCV, in Rotarix and RotaTeq, two U.S. licensed vaccines manufactured by GlaxoSmithKline and Merck respectively, and indicated for the prevention of rotavirus gastroenteritis in infants. The committee will also discuss what additional steps should be considered in this finding. This is a particular matter involving specific parties.

For Topic 2, the committee will discuss and make recommendations on the use of cell substrates, viral seeds and other biological materials used in the production of viral vaccines for human use. This is a particular matter of general applicability.

Based on the agenda and all financial interests reported by members and consultants, no waivers were issued under 18 USC 208(b)(3) and 712 of the Food Drug and Cosmetic

Act.

Dr. Margaret Reynolds will not be attending this meeting. Dr. Theodore Tsai is serving as the industry representative, acting on behalf of all related industry. He is employed by Novartis Vaccines and Diagnostics in Cambridge, Massachusetts. Industry representatives are not special government employees and do not vote.

In addition, there may be regulated industry and other outside organization speakers making presentations. These speakers may have financial interests associated with their employer and with regulated firms. The FDA asks in the interest of fairness that they address any current or previous financial involvement with any firm whose products they may wish to comment upon. These individuals were not screened by the FDA for conflicts of interest.

This conflict of interest statement will be available for review at the registration table. We would like to remind members, consultants and participants that as the discussions evolve, any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement, and their exclusion will be noted for the record.

FDA encourages all other participants to advise the committee of any financial relationships that you may have

with the sponsor, its products, and if known, its direct competitors.

Dr. Stapleton, I turn the meeting over to you.

DR. STAPLETON: Thank you, Christine. I welcome the members to today's meeting. Let's start by asking each member and consultant to introduce themselves and state where they are from. Dr. Cheung, would you like to start, please?

DR. CHEUNG: My name is Andrew Cheung. I am from the USDA Agriculture Research Service in Ames, Iowa.

DR. MC INNES: Pamela McInnes, National Institutes of Health.

DR. WHARTON: Melinda Wharton, Centers for Disease Control and Prevention.

DR. SANCHEZ: Pablo Sanchez, University of Texas, Southwestern Medical Center, Dallas.

DR. GREENBERG: Harry Greenberg, Stanford University.

DR. LARUSSA: Philip Larussa, Columbia University.

DR. DEBOLD: Vicky Debold, National Vaccine Information Center.

DR. GELLIN: Bruce Gellin, National Vaccine Program Office, HHS.

DR. ROMERO: Jose Romero, University of Arkansas for Medical Sciences.

DR. TSAI: Ted Tsai, Novartis Vaccines.

DR. DESTEFANO: Frank DeStefano, Centers for Disease Control and Prevention.

DR. GILBERT: Peter Gilbert, Vaccine Infectious Disease Institute, Fred Hutchinson Cancer Research Center.

DR. HUGHES: Steve Hughes, NCI, Frederick.

DR. BAYLOR: Norman Baylor, Food and Drug Administration.

DR. WEIR: Jerry Weir, Food and Drug Administration.

DR. KRAUSE: Phil Krause, Food and Drug Administration.

Agenda Item: Topic 1: Finding of PCV DNA Sequences in Rotavirus Vaccines

DR. STAPLETON: Thank you. I would like to introduce our first speaker, Dr. Norm Baylor, from Center for Biologics Evaluation and Research, FDA.

Agenda Item: FDA Introduction/Presentation of Issues for Discussion

DR. BAYLOR: Good morning. I am going to provide a background for today's meeting and set the stage for the day.

In February of this year, GSK Biologicals was informed by an independent investigator from the University of California-San Francisco that DNA sequences originating from porcine circovirus were detected in two batches of Rotarix, which is a live attenuated rotavirus vaccine.

GSK initiated experiments to confirm these results, and conduct further investigations. Their tests confirmed the presence of PCV1 DNA in Rotarix in the intermediates, in the production process, the working cell bank, the viral seeds from which the vaccine was derived, as well as the final container.

GSK informed the Food and Drug Administration of the detection of PCV1 DNA fragments in Rotarix. The Food and Drug Administration began its own internal examination and confirmed that the presence of DNA from PCV1 was in Rotarix vaccine.

On March 22 of this year, the FDA recommended that clinicians temporarily suspend the use of Rotarix vaccine while the agency gathered additional information as a precautionary measure.

Although testing by the investigators at the University of California-San Francisco did not find PCV1 DNA sequences in Merck's rotavirus vaccine RotaTeq, the Food and Drug Administration embarked on testing RotaTeq, and recommended Merck do the same. Recently the FDA received information from Merck that preliminary studies identified fragments of DNA from porcine circovirus types 1 and 2, PCV1 and PCV2, in RotaTeq vaccine.

Just a little background on the virus and more of this will be presented later. The porcine circovirus viruses

are small viruses composed of single-strand circular DNA. It is common among pigs, but it is not known to cause disease in humans. There is no evidence at this time that porcine circovirus or PCV1 DNA in U.S. licensed rotavirus vaccines poses a safety risk. To date, no serious or unexpected safety concerns have been identified in postmarketing surveillance of Rotarix or RotaTeq.

GSK, Merck and the Food and Drug Administration continue to investigate these findings of porcine circovirus and/or PCV DNA in these vaccines.

Just to provide a little bit more of an outline of today's presentations, in Topic 1 we will be discussing as per your agenda PCV and rotavirus vaccines. FDA will start out with a safety update on rotavirus vaccines from the Food and Drug Administration. That will be followed by a presentation from the Centers for Disease Control on rotavirus disease and the impact of rotavirus vaccines on rotavirus disease burden in the United States as well as around the world.

GSK will follow and present their current assessment of PCV1 in Rotarix. Our invited speaker, Dr. Gordon Allan from Queen's University of Belfast, an expert in porcine circovirus, will provide an overview of this virus. Then FDA will follow with its current laboratory assessment of PCV1 in rotavirus vaccines.

Then this afternoon we will present topic two, which will cover the advanced analytical methods in characterizing cell substrates. This will be the characterization of cell substrates used in the production of viral vaccines in general for human use, and a summary of these new technologies and we move forward.

There will be no voting questions today. There will be three discussion points. The first discussion point -- and I will give this to you now, and you can think about it as the presentations are being made today, and then these will be presented again at the time of discussion. The first discussion point will be basically information that we will present to you today on the detection of porcine circoviruses for PCV DNA in U.S. licensed rotavirus vaccines. We would like the committee to discuss the available scientific evidence, and identify factors to be considered in assessing the potential risk of using U.S. licensed rotavirus vaccines. We would also like you to discuss any additional scientific studies, perhaps some that we have not covered or the company has not covered, and any other information that you would recommend the Food and Drug Administration consider in its deliberations.

The second discussion point will involve, given the available data about this virus, including the lack of known infectivity as well as pathogenic effects in humans, and that

porcine circovirus or PCV1 DNA may be present in both U.S. licensed rotavirus vaccines, we would like you to discuss factors to be considered in determining whether or what circumstances the benefits of using the rotavirus vaccine outweigh the theoretical risks of PCV.

Lastly, in the second part of today's discussion, we would ask the committee to discuss the application of these emerging technologies and the implications of using these technologies for the detection or known and unknown adventitious agents in vaccines currently licensed as well as those under development.

That is all I have. I guess the next speaker will be introduced. Thank you.

DR. STAPLETON: Thank you, Dr. Baylor. Our next speaker is Dr. David Martin from the FDA, who will speak on the safety update of rotavirus vaccine.

Agenda Item: FDA Safety Update for Rotavirus Vaccine

DR. MARTIN: Good morning. Today I will present FDA efforts to monitor vaccine safety as applied to rotavirus vaccines.

The framework for vaccine safety monitoring has three primary goals, signal generation, signal strengthening, and confirmation of valid associations. Searches of signals include clinical trials during the premarketing phase, as

well as adverse events reported to the Vaccine Adverse Event Reporting System, typically in the postmarketing period. Other sources of signals may include experience with products in other countries where they are licensed.

Signal strengthening and confirmatory studies are a critical second part of the equation. In certain instances, clinical trials are designed for pre-specified safety outcomes, and can serve as a confirmatory source. Indeed, both rotavirus vaccines had a clinical trial design with intussusception as the primary safety outcome.

The Vaccine Safety Datalink serves as an important source of signal strengthening. It is a network of eight managed care organizations that provide a large linked database to the FDA and CDC for analysis.

In addition, industry, academia and government all engage in controlled observational studies which are useful for confirmation of valid associations.

The vaccine safety monitoring system has several strengths. First of all, it is multifaceted. Clinical trials are of course true experimental studies with random allocation of treatment and comparator groups. The Vaccine Adverse Event Reporting System gives us a heterogeneous population from which to solicit rare adverse events that might not be detected by any other means.

VSD rapid cycle analysis gives us near real time

monitoring for mutation pre-specified adverse events. Controlled observational studies give us large populations with real world product use, in which inferences can be made about vaccine adverse event associations.

There are several limitations of the vaccine safety monitoring system. I will highlight a few here. First of all, clinical trials are not typically powered to detect rare adverse events. In VAERs, causal associations usually cannot be determined because we lack denominator data.

The VSD rapid cycle analysis, while occurring weekly, is useful only for generating hypotheses which must then be confirmed through other means. Controlled observational studies, given the fact that they do not have randomization and are not designed as an experimental clinical trial, are vulnerable to bias, like most observational studies. Thus, small increased risks that may be detected could represent this bias rather than a true causal association.

A key limitation that is common to the entire vaccine safety monitoring system is the difficulty we have looking at long latency effects. Obviously clinical trials and observational studies have their outcomes assessed over a period of weeks to months to a maximum of a few years. Furthermore, most reporters to VAERS tend to report events which they temporally associate with vaccination, and so the

greater time that occurs between a vaccination and the event of interest, the less likely that we will receive a report.

RotaTeq was licensed in the United States in February of 2006. Contraindications include hypersensitivity as well as history of severe combined immunodeficiency. I have also added the labeled adverse events from passive surveillance to this slide.

Over 70,000 infants participated in three clinical trials that were submitted to the FDA to support product safety. Serious adverse events were assessed over a 42-day period after any dose. As you can see, there were some differences in overall rates, deaths and Kawasaki disease, but none of these were statistically significant, and these were not pre-specified end points.

There was a large trial with 69,625 individuals which was designed to assess intussusception, and there was no statistically significant association between RotaTeq and intussusception in this population.

Since licensure, 30 million doses have been distributed in the United States and 37 million doses have been distributed globally. Merck sponsored and completed a controlled observational study of approximately 85,000 RotaTeq recipients, and no statistically significant association with confirmed intussusception or Kawasaki disease was found.

Within the Vaccine Safety Datalink, greater than 200,000 doses were administered between May 2006 and May 2008. There was no elevation in risk for intussusception, seizures, meningitis/encephalitis, myocarditis, gram negative sepsis, gastrointestinal bleeding or Kawasaki disease.

Two signals have emerged from VAERS surveillance and literature review. First, a report of secondary transmission has been received and is under evaluation by the FDA. It is also in the published literature. Severe combined immunodeficiency has also been uncovered by rotavirus vaccine administration. By that I mean infants experienced prolonged gastroenteritis and were later diagnosed with having SCID. Thus, a new contraindication was added to the label in December 2009.

Incidentally, a HRSA advisory committee recommendation is currently recommending the addition to SCID to neonatal screening. No other new safety signals have emerged since licensure for this product.

Rotarix was licensed in the United States in April 2008. Contraindications include malformation of gastrointestinal tract that would predispose the infant to intussusception, hypersensitivity and history of SCID. I have also included labeled events from passive surveillance on this slide for your review.

Over 70,000 infants participated in eight clinical

trials submitted to the FDA to support product safety. Serious adverse events were assessed within a 31-day period failing vaccination. Again, overall rates, deaths and Kawasaki disease were presented; none of these were pre-specified end points, and differences were not statistically significant.

A specific trial containing 63,225 infants with intussusception as a primary end point was carried out. Outcomes were assessed 31 days after any dose and 100 days after dose number one, and there were no statistically significant associations with product use. Since licensure, 2.5 million doses have been distributed in the United States; 68 million doses have been distributed globally.

Currently, GSK is carrying out two ongoing controlled observational studies. Outcomes include intussusception, Kawasaki disease, convulsions, lower respiratory tract infections and deaths. VSD rapid cycle analysis is ongoing but less than 5,000 doses have been administered. Outcomes include intussusception, seizures, meningitis/encephalitis, myocarditis, gram negative sepsis, gastrointestinal bleeding, Kawasaki disease and hospitalized pneumonia.

Analysis of all cause hospitalization or ED visits compared with RotaTeq is currently underway in VSD. In VAERS surveillance and literature review, the same SCID issue was

uncovered with Rotarix, with one case as I described previously occurring with this product in comparison to RotaTeq. No other new safety signals have emerged since licensure.

Overall, the components of the vaccine safety monitoring system are complementary. Safety signals for Rotarix and RotaTeq are currently being evaluated in controlled observational studies. Two postlicensure safety signals have been identified for rotavirus vaccines. First is the increased risk posed by rotavirus vaccines to infants with SCID. The second is a case report of secondary transmission with RotaTeq. Postlicensure safety assessment has generated no other safety signals, and multifaceted postmarketing monitoring continues.

DR. STAPLETON: Thank you, Dr. Martin. Does anyone on the committee have any questions for Dr. Martin? If not, then we move to our next speaker, who is Dr. Parashar from CDC.

Agenda Item: Update on Rotavirus Disease and Impact of Rotavirus Vaccines

DR. PARASHAR: Good morning. I was scared for a minute. These slides for circovirus showed up. I'm glad I don't have to speak on circovirus.

I am here to talk to you briefly about the burden of rotavirus disease, and present to you some exciting new

data on the impact of vaccination that has been seen in countries using the vaccine.

Many studies have shown that rotavirus is by far the most common cause of severe gastroenteritis in young children. You can see here this figure in both developed and developing countries; approximately a quarter to a third of severe episodes of gastroenteritis in young children are caused by rotavirus.

The disease manifests as diarrhea and vomiting and fever in some children. The diarrhea and vomiting can be quite profuse, up to 20, 30 episodes of these symptoms per day. That profuse symptomatology can lead to dehydration in young children which if not appropriately managed early can often lead to shock. In settings where treatment of dehydration and rehydration therapy is less accessible, children frequently die from this disease.

Before the vaccine was used or recommended in the United States, rotavirus was an important cause of morbidity in young children. It caused almost three million episodes of illness in children under five each year. That means every child who is born is infected and suffers an episode of rotavirus disease by the time they are five years of age, which results in substantial morbidity in terms of outpatient visits, about half a million outpatient visits each year, and about 55,000 to 70,000 children are hospitalized with

rotavirus each year.

Morality is relatively uncommon in the U.S. because we have access to hydration therapy. Nevertheless, an estimated 20 to 60 children do die of rotavirus each year, and we have some of these deaths that are indeed confirmed by laboratory testing and pathology studies.

The cost of disease is also substantial, about $300 million in direct medical costs. If you include societal cost of loss of caretaker time and productivity, that is almost a billion in the total cost of disease.

If you look at the burden globally, these are the settings where access to hydration is more limited, and rotavirus is a major killer of young children. It accounts for almost half a million deaths in children under five each year. That is about five percent of all childhood deaths. So this is a substantial global burden of mortality, and one of the primary reasons why the vaccines have been developed and have been aggressively pursued.

As was mentioned before, there are two vaccines now available licensed. Both of them have gone through big clinical trials of almost 60,000 to 70,000 infants. As has been mentioned, in these trials that were large, empowered specifically to look at safety signals, there was no increased risk of intussusception or any other serious adverse events. Both the vaccines demonstrated very good

efficacy against severe rotavirus disease in these clinical trials, ranging from 85 to 98 percent.

With these exciting data from the trials, the vaccines have now been recommended for use in many countries around the globe. This is not an exhaustive list. There are countries outside the Americas, several in Europe, South Africa, Australia and a few Middle Eastern countries that are routinely using rotavirus vaccine.

In the Americas, these are the countries. I will show you some early data on vaccine impact. You can see that in the United States, as was mentioned, we have both vaccines currently recommended for routine use. The Merck vaccine RotaTeq is also in routine use in Nicaragua, where it was produced through a donation program from the company. Many countries in Latin America, including countries with large birth cohorts like Brazil and Mexico are using Rotarix vaccine in their routine immunization programs. This vaccine was tested in prelicensure studies largely in Latin America, one of the reasons why these countries began with Rotarix as their rotavirus vaccine in their immunization program.

I will talk to you very briefly about three countries and data on impact of vaccine that has been observed. I will begin with data from the United States. As was mentioned, in the U.S. we had the first vaccine, RotaTeq,

recommended back in February of 2006. In June of 2008 Rotarix was recommended as part of the routine program as well.

I will begin by showing you some data from National Laboratory surveillance. We have a very simple surveillance system, in which we have a network of about 67 laboratories located nationwide. They provide to us on a weekly basis information on the number of tests for rotavirus that they have performed and the number of those tests that were positive for rotavirus.

Here is data from that surveillance system going back from middle of 2000 to early this year in March 2010. You will notice, if you first look at data on the left-side of that arrow for vaccine introduction in 2006, in the pre-vaccine years, each year you will see those sharp winter peaks in the blue line, which are the total number of tests, which corresponds very precisely with the red lines that tested positive for rotavirus. So you see the sharp winter peaks in rotavirus in the pre-vaccine year, which are fairly consistent in magnitude.

If you look at the part in the shaded circle on the right, which is when the vaccine uptake had occurred to a reasonable level, you see a sharp drop-off both in the tests for rotavirus performed as well as the number of tests that are positive, the red line below those blue lines. You will

see in this season in 2010, we are still continuing to monitor the season, but we have almost seen a whiteout of rotavirus activity. So there has been a remarkable and substantial impact on disease in the U.S. after vaccine introduction.

Since these laboratory data do have limitations, we are also monitoring disease trends through an active surveillance network. We have three sites, in Rochester, Cincinnati and Nashville, where they conduct active surveillance. The children coming into the hospital are systematically enrolled and they collect a fecal specimen to test for rotavirus. It provides you with very precise data.

If you look at data from this surveillance system, in the two pre-vaccine season or the season with low vaccine uptake, we had about 45 to 50 percent of children who were hospitalized for diarrhea who tested positive for rotavirus. But if you look at the 2008 data at the bottom extreme right, which is the first season with good uptake of the vaccine, we had a remarkable drop-off. You will see that red bar is now down to nine cases, from 80 to 90 cases, and only six percent positivity from rotavirus. So it really reaffirms that the data from laboratory surveillance are indeed precise and accurate.

We have used these numbers to estimate the national reduction of hospitalizations. It amounts to about 45 to 62

hospitalizations that have been prevented in that season after vaccine introduction. So a big impact in disease prevented.

Another interesting finding and worthwhile to note, through that same surveillance system, we had data on vaccine coverage in the source population of these hospitals.

I want you to focus on the bottom row, which is an age group where as you will notice on the right column, the rotavirus vaccine coverage was negligible, less than one percent of children in that age group were immunized. But if you see the reduction in rotavirus hospitalization rate, it was very comparable to the age groups that were vaccinated. These data and other data that we have suggest that in addition to protecting vaccinated children, vaccine is also likely benefitting unvaccinated children through reduced community transmission of rotavirus, what is typically referred to as herd immunity.

So it is very exciting data, something that we had not anticipated pre-licensure, showing the vaccine has additional benefit beyond its direct product effects.

I will shift focus to a couple of examples from Latin America and begin with data from El Salvador, which is a country where the Rotarix GSK vaccine was recommended back in October 2006 for routine use. You will notice that just like you have seen in the U.S., this is a study where they

looked at hospital admissions for gastroenteritis, and they enrolled these children, tested for gastroenteritis after uptake of the vaccine in 2007. Again it was a low uptake season, but in both 2008 and 2009, two seasons, with good uptake of the vaccine, there has been a sharp reduction in overall diarrhea admissions, the broad red lines, as well as the yellow lines, which are rotavirus positive diarrhea admissions.

These data are very similar to the impact we have seen from use of RotaTeq largely in the U.S., but this is from use of Rotarix in El Salvador. Again, that amounts of reduction of about 70 to 80 percent of rotavirus admissions in this setting.

The last piece of data which I will show you which are remarkable, again an issue that was not looked at in prelicensure studies, is the impact of vaccine on diarrhea mortality. It is hard to do in prelicensure trials, because it would need a huge trial which would be logistically unfeasible. But in routine use of the vaccine, this was a setting where the first look has been performed in Mexico, where they introduced vaccine back in 2007.

Let me orient you to this slide. This is again -- if you remember the curves I showed you for the U.S. from laboratory surveillance, if you first focus on the left of the arrow, the period before vaccine introduction, you can

see that these are all cause childhood diarrhea deaths among Mexican children. Those all cause diarrhea deaths, especially those winter peaks, have been blunted quite substantially the first season with good uptake in 2008, and also in 2009 where that blunting has been sustained.

This is another way to illustrate that the reductions have largely been during the winter months when rotavirus circulates, data from 2007 to 2009. Those red bars are the months during which rotavirus circulates. You will notice, the curves on the top are the pre-vaccine years. That is where the red bars should have been in the post-vaccine years, but you will see there is a wide gap, which is the disease or the deaths prevented from vaccination.

So these are really very exciting data, the first hint that there is impact on mortality from diarrhea from these vaccines, which is very exciting news. I go back to a slide I showed you before; the impact of this vaccine in developing countries, this prevention of deaths, and these data from Mexico reaffirm that that is likely to occur with broader use of the vaccine.

My last slide is to share with you some recent data published earlier this year from a trial of the vaccine in Africa. This is for the GSK Rotarix vaccine. There are data soon to come out for the RotaTeq vaccine from Africa and Asia as well.

One of the questions with the vaccine, since these are oral vaccines, is how well they perform in developing country settings. The vaccine had an efficacy of 49 percent and seven percent in the two settings. But note an important finding in the extreme right column in the circle. Even though the vaccine efficacy was lower in Malawi, 49 percent compared to 77 percent in South Africa, the actual burden of disease prevented in Malawi was more. That is because of the higher background burden of disease. So it illustrates that in developing countries, even if the efficacy may be less than what we are seeing in industrialized settings, these vaccines will have substantial public health impact.

I want to conclude. I hope I was able to show you some data on the exciting impact of these vaccines that hopefully will continue if these vaccines continue to be used.

I do want to acknowledge a variety of ministries of health, our partners at GABI and PATH, who have supported some of these evaluations.

Thank you.

DR. STAPLETON: Thank you. Are there any questions for Dr. Parashar from the committee? If not, I would like to ask Dr. Barbara Howe from GlaxoSmithKline to speak to us and introduce the GSK representatives for today's meeting and their assessment of PCV.

Agenda Item: GSK Assessment of PCV

DR. HOWE: Good morning, everybody. Good morning, Mr. Chairman, members of the committee, FDA, invited consultants, ladies and gentlemen. My name is Dr. Barbara Howe. I am Vice President and Director of North American Vaccine Development, GlaxoSmithKline.

GSK is here today to review available data from our investigations and to share knowledge from experts regarding the unexpected findings of DNA from porcine circovirus type 1 in Rotarix.

For context, it has been approximately eight weeks from the time GSK confirmed PCV1 finding in a validated laboratory until today. The investigation we will present is as exhaustive as was possible in those eight weeks, but testing is ongoing and data are still coming in. However, we do know enough at this point in time to say the following. All available data support that it is a manufacturing quality issue and not a safety issue.

In this presentation, you will see data that supports that PCV1 does not pose a safety risk for infants vaccinated with Rotarix.

To give you a brief regulatory history, Rotarix was first licensed in Mexico in 2004. It was approved in the U.S. in 2008, based on data from 11 clinical studies involving approximately 75,000 subjects over an eight-year

period. This included a study in over 60,000 subjects to assess safety with a special focus on risk of possible vaccine induced intussusception. This is one of the largest studies ever conducted by GSK on any one product.

Importantly, Rotarix was manufactured in compliance with CBER regulations for adventitious agent testing, and in accordance with guidance in place at the time of licensure.

In addition to activities conducted for licensure, extensive postmarketing pharmacovigilance activities and studies are ongoing worldwide.

Rotarix has been licensed in over 110 countries, as shown in green on this slide. More than 69 million doses have been distributed globally, two and a half million in the U.S. It was the first rotavirus vaccine to be granted pre-qualification by the World Health Organization. This allows international agencies such as PAHO and UNICEF the much-needed access to Rotarix for mass vaccination programs.

As you have heard, rotavirus is the leading cause of severe childhood diarrhea in developed and developing countries, resulting in over half a million deaths every year. An introduction of vaccination has already led to substantial reduction in rotaviral gastrointestinal disease and death worldwide, including in the U.S.

Recently Rotarix was one of mutation products reviewed at a pediatric advisory committee. The PAC

unanimously agreed with CBER's conclusion that no new safety concerns were identified for Rotarix, and with a recommendation for continued routine monitoring.

The PAC didn't specifically review the product with the knowledge of PCV1 because the data were just becoming available at that point in time. However, what we have since learned is that the product reviewed contained PCV1, as it has since the beginning of development.

As soon as GSK confirmed that PCV1 DNA was in the product, we immediately began collaborating with experts in porcine viruses and analytical detection methods in order to further investigate the finding. We looked for a precedent to guide our investigation.

As you know, this is not the first time an adventitious agent has been found in the vaccine. We used the experience of avian leukosis virus, which was found in a vaccine in the 1990s, in order to guide and inform our investigation algorithm for Rotarix. The investigation was specifically designed to address the following: The source, the nature and the amount of PCV1 in the manufacturing process, the clinical implications and potential remedial actions.

It is important to note that we didn't just limit our investigation to Rotarix. Since GSK's inactivated polio vaccine or IPV utilizes the same master Vero cell bank as

Rotarix, we also tested IPV containing vaccines. As with Rotarix, PCV1 DNA was detected early in the IPV manufacturing process, but unlike Rotarix it was not found later in the process or in the final containers. This is likely due to the purification and inactivation steps used in the manufacture of an inactivated vaccine such as an IPV.

We also tested all other GSK vaccine cell banks other than Vero cells, and no PCV1 was detected. Therefore, the investigation that I will be describing today will largely focus on Rotarix.

As data has become available from our investigation, GSK has and will continue to share them with regulators and global health care agencies, including the European Medicines Agency, WHO and FDA. The agencies agreed that PCV1 is not known to cause any illness in humans or animals, and Rotarix has been studied extensively before and after approval with the presence of PCV1 in the vaccine posing no risk to human safety.

While FDA has recommended temporary suspension of Rotarix in the U.S. as a precautionary measure, most countries where rotavirus frequently causes severe illness and death have chosen to continue to use Rotarix pending further investigation because of the tremendous need and benefit that we just heard about from Dr. Parashar.

Let me just take you through our presentation

agenda. First of all, we will have Dr. X.J. Meng, who is Professor of Molecular Virology at the College of Veterinary Medicine at Virginia Tech. He will provide an overview of PCV1. Dr. Meng is a recognized expert in porcine circoviruses. His research focuses on the molecular mechanism of viral replication and pathogenesis as well as veterinary vaccine development. He developed the first USDA licensed vaccine against porcine circovirus.

Next we will have Dr. Emmanuel Hanon, who is Vice President of Early Research and Development, who will provide results to date from our manufacturing investigation. Then we will have Dr. Gary Dubin, who is Vice President of Global Clinical Development, and he will present the results of our clinical investigation to assess the possibility of PCV1 infection of vaccine recipients. Then Dr. Leonard Friedland, who is Vice President of Clinical and Medical Affairs, will present Rotarix safety, efficacy and overall benefit-risk. Then I will come back to conclude our presentation and discuss next steps.

We also hope to have an additional expert here today to help answer questions. Dr. Hans Nauwynck, who is the head of the Laboratory of Virology and Professor of Veterinary Medicine at Ghent University, is a PCV expert whose lab is responsible for running the immunopyroxidase assay in support of our manufacturing and clinical

investigations that you will hear about more in a moment. But unfortunately his plane was diverted to Newark this morning, and he is currently coming via ground transportation, so we are hoping he will be here by the Q&A period.

I will know turn the podium over to Dr. Meng.

DR. MENG: Thank you, Dr. Howe. Good morning, everyone. I am here today to present to you some of the background information regarding the PCV1 virus that is relevant to this meeting.

As Dr. Howe mentioned, I have been studying this virus since 1999. I have been published extensively on both PCV1 and PCV2.

Now, PCV1 was initially discovered as the cell culture contaminant of the porcine kidney cell line PK-15. The virus belongs to the family *Circoviridae*. As you can see from this slide, PCV1 is a small, non-enveloped virus with an icosahedral symmetry. The genome of the virus is single-stranded, circular DNA molecule of about 1.7 kb in size.

There have been several studies evaluating different methods of PCV1 inactivation. It has been shown that PCV1 is resistant to inactivation in the pH3 conditions and also by chloroform. It has also been shown that PCV1 is resistant to heat inactivation at 70 degrees for 15 minutes and 60 degrees for 30 minutes. So therefore some products if

not done properly may still contain infectious PCV1.

These vaccines have been shown to have variable success in PCV1 inactivation. For example, treatment with Nolvasan and ethanol have no significant titer reduction. Other disinfectants such as sodium hydroxide and bleach are effective with about a two to four log titer reduction.

It is important to know that PCV1 is not the PCV2 virus that is associated with pig disease. As you can see from these slides, the two viruses are genetically distinct and they belong to two separate clusters. These two viruses by approximately 25 percent nucleotide sequence, and importantly they also differ in their ability to cause disease. PCV1 is not known to cause disease, whereas PCV2 is associated with postweaning multisyndromic wasting syndrome in pigs. However, neither virus is known to cause disease in humans.

It has been shown that PCV1 replicates and causes a productive infection in porcine kidney cell lines PK-15, even though the virus replicates in relatively low titers. Mankus(?) group in Germany evaluated several human cell lines for their susceptibility to PCV1 infection. They found that PCV1 caused non-productive infection in three human cell lines, as shown here in this slide, the 293, HeLa and Chang liver cell lines. They detected a viral gene expression in these human cell lines. However, infectious viral particle

was not produced, and the supernatant from the infected cells were not able to infect naive cells. Also, there was a report of PCV1 infection in human blood leukocytes, some virus-like particles were visualized by electron microscope in the cells, and the PCV1 DNA was detected by PCR. However, it is not known if infectious virus was generated, because the authors did not perform infectivity assay in these studies.

It is known that PCV1 causes widespread infection in pigs. For example, about 60-95 percent of pigs tested in Germany and 26 to 55 percent of pigs tested in Canada are positive for PCV1 antibodies. It has been shown that pigs experimentally infected with the PCV1 remain clinically healthy. There is no pathological lesions in those infected pigs.

Gordon Allan's group in Belfast conducted the first PCV1 pathogenesis study in cluster deprived pigs. They detected PCV1 antigen in a variety of tissues for nine days, but the infected pigs did not develop disease.

In 2004, my lab conducted another PCV1 pathogen study in conventional pigs. Again PCV1 was detected in the blood for up to 35 days. However, we did not observe pathological lesions in the variety of the cases we examined.

Therefore, the available data indicates that the PCV1 is not pathogenic. In fact, the non-pathogenic virus,

the PCV1 is used as a vaccine vector in the first USDA fully-licensed kill vaccine against PCV2.

So far there is no credible evidence suggesting human infection by PCV1. There was one early report of a PCV1 antibody detection in humans in Germany. However, the authors showed that when the human sera were frozen and thawed repeatedly, and when the serum samples were stored at 40 degrees, they lost binding specificity to the PCV1 virus antigen. Also they showed that the particular optical density values from human sera was lower than those from the pig serum, indicating that antibody detected in that study were likely to be due to cross reactivity with another agent.

In addition, subsequent studies could not reproduce the result of this initial study. For example, in 2000, Gordon Allan's group did not find the PCV1 antibody in 120 humans tested in Belfast. Also in 2000, John Ellis from Canada and Gordon Allan from Belfast tested 50 swine veterinarians who had contact with the pigs, and also six lab workers who had worked with the porcine circovirus, and 33 normal blood donors for PCV1 antibody, and all of them were negatives.

Then in 2004, Annette Mankertz' group from Germany tested a large number of human serum lymph node and urine samples, including 168 samples from immunocompromised patients, and again all inactive for PCV1 DNA.

I would like to mention here that the detection of PCV1 in commercial products including vaccine is not new. This has been reported before. For example, in 2004 my lab detected a PCV1 DNA in commercial pepsin. However, the viral DNA in the pepsin was not infectious when inoculated into the PK-15 cell lines or inoculated into piglets.

Quintana's group in Spain tested some of the swine vaccines on the market, and they found that about 11 percent of the porcine vaccines are positive for PCV1 DNA.

Just this year, Eric Dauber's group, the same group that found the PCV1 DNA in the Rotorix, tested a small number of pork products in the United States, and they found that about 69 percent of them contained either PCV1 or PCV2 DNA. They also found that about five percent of the human stool samples collected in Minnesota had a detectable PCV1 or PCV2 DNA. The authors believe that this finding may reflect dietary consumption of pork products.

In summary, the PCV1 infection is widespread but does not cause disease in pigs or other species, including human. There is no credible evidence of a human infection by PCV1. The virus has been detected in commercial products, including veterinary vaccines, in pork products and also in human stool in the United States.

In conclusion, this is a non-pathogenic virus. It is common in pigs and pork products. Even when present,

would not be a cause for safety concern, because we are likely exposed to this virus on a daily basis through the consumption of pork products. The bottom line is that this virus is not known to cause disease in pigs or in humans.

Thank you. Now I am going to turn the podium to Dr. Hanon.

DR. STAPLETON: I think it would be good to have questions now, actually. It will be nice while the topic is hot. Thank you. Dr. LaRussa.

DR. LARUSSA: Just one question about the two human studies that you mentioned, the Hattermann study, where they looked for DNA and then the last study you mentioned, the Li study, where they looked for DNA by PCR for PCV1.

In either of those studies, in the first study, did they actually look at stool samples? And in the second study, did they look for live virus?

DR. MENG: They did not look for a stool samples. In the first study they looked at the blood for antibody. In the second study they looked at tissue sample and blood for DNA. So they did not look for the stool for the virus.

DR. LARUSSA: So just to be clear, in the second study where they found DNA in the stool, they didn't see if that was live infectious virus?

DR. MENG: No, in the second study they looked in blood, urine and lymph nodes. They did not have the stools.

DR. LARUSSA: I am talking about the Li study.

DR. MENG: That study did look at the human stool samples. They did find the PCV1 and PCV2 DNA sequences.

DR. LARUSSA: Right, but did they look for live virus in the stool in that study?

DR. MENG: They did not perform any additional study. They only found the DNA -- they did not do any infectivity study, so we do not know if there was infectious virus in the stools.

DR. GREENBERG: Thanks for the presentation. I have two questions, one for you. The lack of pathogenicity of PCV1 in normal pigs or apparently in humans exposed to it, is interesting. Have people done experiments with immunosuppressed pigs or have people looked at immunosuppressed people who might have been exposed to PCV1?

DR. MENG: In terms of animal studies in pigs, as far as I know, I don't believe anybody has looked at any immunosuppressed pigs, in terms of their ability for PCV infection, no.

DR. GREENBERG: My second question was for Dr. Howe. That is, since you mentioned it was found in your rotavirus Vero cell grown virus and your inactivated polio, do we take that to mean that your stock Vero cells are carrying PCV1 or is it just specific to those two viral vaccine preparations?

DR. HOWE: I actually suggest we go on with the presentation, because we are now going to move to the manufacturing investigation, and we can cover it during that presentation, okay?

DR. STAPLETON: Dr. Gellin, you had a question?

DR. GELLIN: Your suggestion about cross reacting antibodies, any theories on what that may have been cross reacting to?

DR. MENG: It is probably a related but different agent. We don't know. If you look at the study, it is in a way -- I don't know how to say this, because normally when you have frozen a serum sample you don't lose the binding specificity. But in this case they did, so that indicates something isn't right about the antibody they detected. The hypothesis is that it is probably with some other agent that has shared antigenic appetite with the circovirus.

DR. ROMERO: A follow-up question to Dr. Greenberg, which I was going to save for later. Has anybody looked at the stool from individuals that are known to be shedding rotavirus vaccine strains at this time to see if there are circovirus in there?

DR. HOWE: Again, I think it would be best perhaps to move through -- at least through the GSK investigation, the manufacturing and clinical investigation. Then we can answer these questions.

DR. ROMERO: I had a couple of technical questions for Dr. Meng. Is there an infectious clone of PCV1?

DR. MENG: Yes, there is.

DR. ROMERO: And if you transvect that DNA, does it replicate in human cells?

DR. MENG: Nobody has looked at human cells yet, but in pig cells, yes.

DR. ROMERO: And do PCV1 and 2 recombine?

DR. MENG: I don't believe so. At least no data available so far indicate that.

DR. ROMERO: Two quick questions. Is the receptor for PCV1 known, and is there a human homolog?

DR. MENG: The receptor is not known.

DR. STAPLETON: One last question.

DR. CHEUNG: There is a report from Canada that there is a recombination between PCV1 and PCV2. That is virus isolated in Canada that actually showed that.

The second thing about the second study on the stool, I don't think the authors are really that clear about transmission from finding the PCV DNA in the stool. In their conclusion they not say that it comes from consumption of meat, nor did they say that it is no replication in humans. So I think the statement that there is no infection in humans, I think that is premature.

DR. MENG: Yes, but Dr. Cheung, you can look at Dr.

Ellis' study, they tested only 13 pork products, so it was a very small number, and they found nine of them were positive for the PCV1 and PCV2. Also they tested some human stool sample, and there was about five percent positivity.

So it is their conclusion, not mine, in their paper they indicate the detection of PCV1 in human stool is likely from dietary consumption of contaminated pork products.

DR. STAPLETON: Are there any other questions for Dr. Meng? If not, we will move ahead.

DR. HANON: Good morning, Mr. Chairman and members of the committee. I am Dr. Emmanuel Hanon. I am Vice President responsible for early development of prophylactic vaccine induced biologicals. I will present GSK's manufacturing investigation three-year divided discovery of the PCV1 DNA in Rotarix vaccine.

Our investigation was designed in agreement with the FDA to determine the source and nature of PCV1 material found in Rotarix and what it means for human infants. I will begin by explaining how Rotarix is manufactured, where we found the PCV signal, and what we believe is the root cause of its presence.

The routine manufacturing process for Rotarix is based on the propagation of rotavirus viruses on a Vero cell line with porcine derived trypsin to activate rotavirus vaccine. It is important tools that as part of initial

licensure, the cell bank, viral seed and porcine derived trypsin were all tested and found to be free of advantageous agents. This testing was done in compliance with U.S. and international regulation and guidance.

To produce each batch of vaccine, Vero cells are expanded, infected with the working viral seed and further incubated for viral expansion. The viral harvest is then collected and run through a purification process that includes a DNAase treatment followed by formulation and filling into final container.

Turning now to our recent vaccine, when we screened for PCV1 DNA using quantitative PCR, we found that the final container, the purified bulk, the viral harvest, the viral seed and the Vero cell line all contained PCV1 DNA. Full sequencing confirmed 98 percent identity of the PCV1 genome and no PCV2 DNA sequence was detected. So all the PCV1 originated in the Rotarix viral seed as well as the Vero cell line.

To explain this, I need to take you back in time before Rotarix development began to GSK's source, the original Vero cells, in the 1980s from which we produced the master cell bank in 1983 and the working cell bank in 1993. The working cell bank was used to produce the Rotarix viral seed in 1999 from an ancestor seed produced on an alternative cell line.

When screening for PCV1 DNA was performed using Q-PCR, the Rotarix virus seed, the working cell bank and the master cell bank were all found to contained PCV1 DNA. In contrast, the Vero cell line source from ATCC and the ancestor seed were negative. So we speculate that the PCV1 might have come from porcine derived trypsin used to propagate cells during the production of the master cell bank. Note that trypsin was not routinely irradiated when the master cell bank was generated.

So based on these findings, you can see that redeveloping a Rotarix free of PCV1 would be a complex process that would involve generating a new cell bank and a new Rotarix viral seed as well as conducting necessary clinical trials in agreement with the authorities, and this will take several years.

We have seen where the PCV1 signal can be found and where it comes from. I will now describe the nature of the signals. As Dr. Howe mentioned, our investigation followed the same testing algorithm as the one used to investigate the presence of avian leukosis virus in a commercial vaccine in the 1990s.

We addressed the four questions seen on this slide.

For the first question, whether intact virus as opposed to DNA fragments was present in the Rotarix

manufacturing process, I won't describe or explain in detail to save time, rather I will just tell you our bottom line result. We cannot exclude that at least a fraction of the PCV1 DNA might be present in the form of viral particle.

Now, let me be clear that this does not necessarily mean that this viral particle that is present would be capable of infecting cells. This issue is in fact addressed by the second question: Do we find viral particle in the Rotarix manufacturing process capable of infecting permissive cells? By permissive cells, I mean for example cells from the natural host.

To answer this question, we ran an in vitro infectivity assay. This assay is based on the use of the permissive porcine kidney cell line, PK-15. When these cells are incubated with the PCV1 virus they become infected and initiate viral gene expression that can be detected by RT-PCR.

The negative and positive control shown here validates the ability of the assay to detected infective viral particles, we know with a high degree of specificity and sensitivity. It can detect down to one to ten $TCID_{50}$ per test. In fact, we did try alternative testing methodologies such as quantitative PCR to measure accumulation of viral DNA, but this led to inconclusive results. We could not discriminate inoculum PCV1 DNA from newly synthesized viral

DNA.

So in order to investigate the presence of infective viral particle in the Rotarix manufacturing process, we tested the equivalent of 1500 Rotarix doses using Rotarix purified bulk, which is as I'll show you the last step in the production before the final container. We detected messenger RNA, indicating the presence of infective viral particles.

To confirm this result, we repeated the test with the equivalent of 300 doses of Rotarix from purified bulk. Again, the test was positive. We reached the same conclusion when using Vero cells as an alternative permissive cell line.

So at this point we knew we had virus that could infect permissive cells. Now we needed to know how much.

For that we ran an additional assay to estimate the titer of infected viral particles. In this assay, the sample is incubated on cells at different dilutions, then cells are further incubated for seven days and processed to detect expression of PCV1 viral protein by immunostaining. We ran the assay on the viral harvest in the manufacturing stage containing the highest amount of DNA copies, ten_{10}, in order to maximize options of detecting infective viral particles.

This titration assay demonstrated the presence of 10_2 $TCID_{50}$ per milliliter in the viral harvest. Based on these results, and assuming no impact of the downstream

manufacturing process, we can also estimate that there may be a low level of infected viral particle in the final container, the equivalent of three $TCID_{50}$ per dose. Because this is only an estimation, we will perform additional experiments to determine the precise number of infected viral particles in the final container.

At this point in our investigation, we knew we had PCV1 virus in the Rotarix manufacturing process that can infect pig cells. But pigs are not humans, so what does it mean for humans?

To address this question, we first looked at whether PCV1 virus present in Rotarix is able to produce infected viral particles in human cells. In fact, at least from the PCV1 virus, this has been already investigated by Hattermann et al.

Let me explain the principle of the assay used. Consider first a PK-15 cell. When incubated with PCV1 virus, these cells undergo viral gene expression and produce progeny infectious viral particles in a supernatant, which can be detected with a classic infectivity assay. But would the same thing happen with human cells?

To find out, Hattermann et al. tested 18 human cell lines, and this is what they concluded. Although PCV1 gene expression and DNA replication took place in human cells, the infection is non-productive.

We also performed our own investigation to confirm these results. We tested three human cell lines, MRC5, a diploid cell line, U937, a monocytic cell line, and the Hep2, transformed human cell line. We included in these tests the PK-15 cell line as a positive control, because we already knew PCV1 can undergo productive infection in these permissive cells.

Here we are looking at two things. One, could the PCV1 virus induce viral gene expression in the cell line tested and two, could this result in the production of infectious viral particles.

As expected, we observed both viral expression and productive infection using PK-15 cells. With the MRC5 and U937 cell line, we did not observe viral expression, nor did we see productive infection. With the Hep2 cell line, results were also consistent with the Hattermann paper. There was detectable viral expression, but again no productive infection. Importantly, we obtained exactly the same results using Rotarix purified bulk, which as we know contains PCV1 viral particles.

So at this stage, the evidence we have does not indicate that PCV1 associated with Rotarix can induce productive infection in human cells.

I have shown you the results of Rotarix manufacturing investigation. But we conducted also a similar

investigation on other GSK products. First we tested five other cell lines used in GSK, and all were shown to be negative for PCV1 DNA, in contrast to the Vero cell line.

Second, we tested the inactivated polio virus containing vaccine, which utilized the same master cell line as Rotarix. Before I present the results, it is important to note that the production process for polio containing vaccine is very different from the Rotarix process. There is a specific purification step using chromatography, as well as an inactivation step using formaldehyde.

Here are the results of the investigation for polio containing vaccine compared with the Rotarix results. In contrast to Rotarix, in the polio containing vaccine PCV1 DNA was detected only in the viral harvest. We saw a much lower number of DNA copies per milliliter than in Rotarix, ten to the five-fold less. PCV1 DNA was not detectable in the purified bulk, in the inactivated bulk nor in the final container. Note that we tested the polio vaccine final container, that does not contain aluminum salts, which interfere with testing, as we have seen for chemrix and pediatrix(?) final container.

More importantly, using the PK-15 infectivity assay, we also tested the equivalent of 1500 doses from both the purified and inactivated bulk. No infective viral particles were detected. Let me remind you that the PK-15

infectivity assay we developed at GSK is able to detect down to one to ten $CCID_{50}$ per test.

These results can be understood based on the following. First, we estimated a PCV1 DNA clearance factor of at least ten_4 for the purification step. This was estimated running the Rotarix viral harvest through the polio virus purification step.

Second, be aware that the inactivation procedure is based on incubation of two weeks at 37 degrees in a solution containing ten percent of formaldehyde. It has been published that sero viruses are not insensitive to formaldehyde.

GSK will conduct additional investigations to quantify precisely the clearance potential of the purification and inactivation step on PCV1. But at this point, we can conclude that no PCV1 DNA and no infective viral particles can be found any time after the purification step in the polio containing vaccine.

To conclude my presentation of the manufacturing investigation into Rotarix. First, GSK is in the process of evaluating a manufacturing change to remain in compliance with regulation. Second, PCV1 signal associated with Rotarix and after having done this wall testing algorithm, we cannot exclude the possibility of low level of PCV1 viral particle capable of infecting pig permissive cells in final

containers, but at this stage there is no evidence of productive infection in human cells.

This was also true in the case of the avian leukosis virus case in the '90s, but this is in vitro evidence, and this is not enough at this stage.

As was done with the avian leukosis virus, we must also answer the fourth question, which is an important question: Is PCV1 capable of causing infection in human infants? Dr. Gary Dubin will present the result of that path of our investigation.

Thanks for your attention.

DR. STAPLETON: Dr. Hanon, Dr. LaRussa has a question for you.

DR. LARUSSA: Could you be a little bit more specific about what happened in the Hep2 cell lines? You said there was evidence of viral expression. Was that transcripts or was there actual protein made?

DR. HANON: The assay that we used was a reverse transcription PCR for the REPP gene, so it is an expression of messenger RNA, and the signal was definitely positive. We didn't have time to run additional experiments to detect the expression of protein.

In the Hattermann paper, I think he also confirmed by messenger RNA expression. I don't remember if additional experiments were done to investigate the protein expression

as well as DNA replication.

DR. GREENBERG: Do you know how sensitive the PK-15 cell line is compared to, let's say, infant pig inoculation in many infectious diseases? The cell culture turns out to be substantially less sensitive than the natural host. I have no idea; I am just trying to get a feeling of what is the most sensitive assay for infectivity.

I have a second question after you answer that.

DR. HANON: To my knowledge there is no available data comparing the piglets approach versus the in vitro infectivity assay. What we did, as you have seen, is, we chose to select the stage in the manufacturing process allowing us to test several thousand of the equivalent of final container doses. So using that approach, we feel relatively comfortable in the ability of the test to detect very low amounts of PCV1 viral particle, so all internal investigation indicated that we can detect for each test an equivalent of one to ten $TCID_{50}$ of infective PCV1 viral particles.

DR. GREENBERG: I simply meant that the estimate of how much infectious virus is in a dose of Rotarix could be different if your in vitro assay underestimates actual infectivity. But you will find that out.

The other question I have is have you had the opportunity to look at primary human cells as opposed to

human cell lines? Frequently the infectivity of viruses varies very much.

DR. HANON: If I may, we didn't do that. We are in the process of doing that. But I can refer again to the Hattermann paper that tested human-pig cells that have been submitted to that testing, and led to the same conclusion. Note that in pig, it is this cell type that is highly infected with PCV virus.

DR. STAPLETON: Dr. Coffin, I should have you introduce yourself and say you were unavoidably delayed, I understand.

DR. COFFIN: Yes. I'm sorry, I wasn't able to make it on time. I am John Coffin. I am with the faculty at Tufts Medical School, in the Department of Molecular Biology and Microbiology. My expertise is largely in retroviruses, but in virology generally.

The question I have, maybe this was extractable from your presentation, is, what is the specific infectivity of the virus in infectious units per DNA molecule? And does that change through the purification process?

DR. HANON: I cannot answer the second part of your question. What I can answer is the first part. We are performing investigation trying to estimate the difference of the ratio between the number of DNA copies and the number of infected viral particles in a given suspension. We end up

performing such analysis with a very high ratio. So you have much more DNA copies that infect the viral particle. That ratio can be up to ten_7.

DR. COFFIN: Do you know if that is typical for virus preparations that are grown under sub-optimal conditions? Or is your virus substantially defective relative to what a virologist would call a good virus preparation?

DR. HANON: What I can tell you is that in interaction with *Circo* viruses experts, they told us that this is not an unexpected finding for *Circo* viruses.

DR. ROMERO: Perhaps you already answered this question with that last comment. In Victoria's paper, the one that was published in *JVI*, they estimate on average a viral DNA copy per vaccine dose of 2.25 time ten_5. So you have three infectious units. So you are saying that you have a substantial number of defective interfering particles?

DR. HANON: You mean defective viral particles?

DR. ROMERO: Yes.

DR. HANON: First, I think it is important to mention that the three $TCID_{50}$ is an estimation, so it is not something we have been able to measure. We simply take into consideration the dilution factor within the manufacturing process. But this indeed would imply that again, we have a very high number of DNA copies,, but very few of them are

indeed infective.

DR. STAPLETON: Dr. Hughes, did you have a question?

DR. HUGHES: Actually, my question was exactly the same question that John Coffin asked.

DR. STAPLETON: Any additional questions for Dr. Hanon? The committee will move ahead.

DR. DUBIN: Good morning, members of the committee. My name is Dr. Gary Dubin. I am responsible for the global late clinical development programs at GSK Biologicals. Over the next few minutes, I am going to present an overview of the clinical investigations we performed in response to the detection of PCV1 DNA in Rotarix.

As you have just heard in the previous presentations, PCV1 is not known to productively replicate in human cells or cause disease in humans. However, as part of our clinical investigation strategy, we evaluated whether PCV1 signal in Rotarix is associated with the presence of virus capable of causing infection in infants. To do this, we performed blinded retrospective laboratory testing using archived clinical samples collected in completed Rotarix clinical trials. The studies we selected were required to be placebo controlled and to involve the collection of both pre and post vaccination sero and stool samples at predetermined time points.

We identified four completed studies which were conducted in Africa, Asia, Europe and Latin America. In all four of these studies, infants received their first dose of Rotarix between six and 12 weeks of age. In three of the studies, infants were required to be healthy at study entry, and in one of the studies we specifically evaluated administration of Rotarix to HIV positive infants. This study was included in the evaluation because it might be speculated that PCV1 replication if it were to occur in humans might be enhanced in an HIV positive population.

Our goal was to test samples from 20 subjects in each of these four studies for a total of 80 subjects. However, for one of the studies a limited number of samples were available, and therefore we compensated by increasing the size of the subset in another of the studies to maintain the total sample size of 80 subjects.

Our clinical testing had two objectives. First, we evaluated the presence of PCV1 DNA and the pattern of detection in stool samples collected at predetermined time points after a single dose of Rotarix with placebo. Secondly, we evaluated whether subjects receiving two or three doses of Rotarix or placebo developed an immune response to PCV1, as assessed by the presence of antibodies against PCV1 detected post vaccination.

In each of the studies, we selected a subset of

samples for testing using the following criteria. We first considered the subject enrollment number, with the goal of selecting for testing the first ten Rotarix and ten placebo recipients enrolled in each of the studies with adequate samples.

The adequacy of samples was assessed according to two requirements. First, the availability of pre and post vaccination time points, and additionally sufficient residual volume in each sample to allow testing.

This table provides an overview of the clinical studies we selected for sample testing, highlighting in particular the number of subjects in each study, the countries in which the studies were conducted, the study populations, the number of doses administered, and the timing of post vaccination samples.

As you will note, in each study a two dose vaccination schedule was used, except in a study performed in HIV positive children, where three doses were given. Stool samples were collected on at least two time points, post vaccination for evaluation of PCV1 DNA, for observation periods of up to 45 days post vaccination. Serologic testing was performed on the samples collected after administration of the last dose of Rotarix.

To preserve the integrity of the evaluations, given that these were retrospective evaluations, all laboratory

analyses were performed by blinded laboratory personnel. For the analysis of stool samples we performed quantitative polymerase chain reaction or Q-PCR to detect PCV DNA in two replicates from each sample. For detection to be considered positive or negative, the replicates had to provide concordant results. If the results of the replicate testing were not concordant, as shown in these examples, a third replicate was tested and the final result for the sample was determined according to a decision algorithm.

The final result of each sample was alternatively reported as positive, negative or inconclusive. If the Q-PCR result was positive or inconclusive, DNA sequencing was performed to confirm the identity of the amplified DNA as PCV1.

For the analysis of serum samples, an ImmunoPeroxydase Monolayer Assay was used to detect anti-PCV1 antibody responses. The assay uses PCV1 infected PK-15 cells, and was developed by Professor Hans Nauwynck at Ghent University. Professor Nauwynck also performed all the serologic testing for these evaluations.

This assay has been previously used to detect anti-PCV1 in pig serum, and was adapted for testing of urine serum samples. As you heard from Dr. Meng, there is no credible evidence of PCV1 seropositivity in humans, which meant as a result there were no human reference sera available. So for

this reason, the serologic assay uses pig serum as the positive control. At the bottom of this slide, you can see examples of monolayers considered as positive or negative, and the two patterns are easily distinguishable.

Just a few words about our analytic approach. The analytic approach was pre-specified and involved descriptive analyses without any formal hypothesis testing. The sample size was selected based on the feasibility of generating data in time for review by this committee. So as soon as the PCV1 issue emerged, we rapidly formulated a testing plan and began the process of identifying studies for retrospective testing where we had appropriate samples, and also the process of identifying assays. This left us approximately three weeks for assay setup and sample testing.

It should be noted that because dietary or environmental exposure to PCV1 may occur, the interpretation of PCV1 DNA patterns in stool needs to be carefully considered. This slides shows some hypothetical examples of possible detection patterns and interpretations.

The first pattern shown in this slide is a subject, and this is hypothetical, who is negative at pre and post vaccination time points, which could be interpreted as indicating no evidence of viral replication. The next pattern which shows PCV1 DNA at pre and one post vaccination time point might be interpreted as evidence of dietary

exposure to the virus.

Another possible pattern is a positive result at only the earliest pre vaccination time point, which might be interpreted as evidence of transient passage of the PCV1 DNA present in Rotarix through the gastrointestinal tract. There are many other possible patterns which might be considered.

But most importantly, the pattern which we believe would be consistent with evidence of viral replication, which is shown at the bottom of this slide in yellow, is a pattern where the pre vaccination sample would test negative, the first post vaccination sample would be positive, and then a higher level of DNA positivity would be observed at subsequent time points.

In any case, beyond looking at the patterns of DNA detection, the DNA results must be interpreted in light of the serologic results for each individual subject.

I would now like to move on and present the results from the studies. This slide shows the results of stool and serology testing from studies one and two. The table shows the number of infants testing positive for PCV DNA or with inconclusive results, and those results are shown in parentheses at each time point.

In each of the studies, a single infant tested positive for PCV1 DNA at day seven post vaccination. This was the earliest time point evaluated in these studies. Both

infants were in the Rotarix group, and the day 15 samples in each infant tested negative. The DNA sequence analysis in each of these infants confirmed that the PCV DNA detected was identical to the PCV1 sequence present in Rotarix. One infant in the study had an inconclusive Q-PCR result at day seven.

As I indicated previously, this pattern of DNA detection at only the earliest time points suggests transient GI tract passage of PCV1 DNA present in Rotarix. This conclusion is supported by the absence of PCV1 antibodies in the post vaccination serum from all infants tested in these studies, as shown on the right-hand side of the table where you can see the pre and post vaccination serologic results and no subjects seroconverted, including those that were PCV1 DNA positive.

The results of stool and serology testing from studies three and four are summarized on this slide. In study three, two infants in the Rotarix group had PCV1 DNA detected at day three. In one of these infants, the day seven sample was inconclusive, and in the other infant, the day seven sample was negative. DNA sequences at day three were confirmed to be the sequence detected in Rotarix. In other words, the DNA sequence was identical, 100 percent sequence identity to the sequences present in final container material.

Of note, none of the infants who received Rotarix demonstrated PCV1 serum conversion. Again, this pattern of DNA detection at only the earliest time points suggests transient GI tract of the PCV1 DNA present in Rotarix.

You will also note that in the placebo group of study three, one infant had PCV DNA detected by Q-PCR at day 15, but DNA sequence analysis was negative and today we have not confirmed the presence of PCV1 sequences.

Two other infants in the placebo group had inconclusive PCR results at day 15 and 30.

In study four, which is shown on the bottom table on this slide, and this study again enrolled HIV positive infants, PCV1 DNA was not detected in any infants who received Rotarix. So seroconversion was not detected in Rotarix recipients, and this is shown on the right-hand side of the table. These subjects had serologic testing performed after receiving three doses of Rotarix.

This table provides an overall summary of the PCV DNA testing patterns in Rotarix recipients who had a positive or inconclusive result at at least one time in point. As you can see, of the 40 infants included in the evaluations, in total four had positive findings on either day three or day seven. One additional infant had inconclusive results at day seven.

Of note, there were no infants with positive or

inconclusive results at any of the later time points evaluated in these trials. Again, in each individual infant who had a positive result, the pattern was consistent with transient passage of DNA, since the positivity was detected only at the earliest time points post vaccination.

Now, because PCV1 has not been associated with clinical disease in human, there is no specific adverse event profile which might be expected to be associated with PCV1. Nonetheless, to be complete, we reviewed the clinical data in the five infants who had a positive or inconclusive PCV1 stool test result for adverse events that are solicited in our Rotarix trials. As you can see, none of the infants had diarrhea, and the overall pattern of solicited adverse events in these infants who had PCV1 detected was similar to the pattern observed in placebo recipients, as prescribed in the U.S. prescribing information.

Let's summarize the data that I have just presented, which provides no evidence for PCV1 infection in the infants evaluated. PCV1 DNA was detected at only the earliest time points in these studies, consistent with transient passage of the DNA present in Rotarix through the GI tract. The sequences detected in the stool in the four infants testing positive are identify with 100 percent sequence identity to the PCV1 sequence in Rotarix. One might expect that nucleotide changes would occur during the

replication process if replication were occurring in human blood cells.

Lack of infection is further supported by the absence of seroconversion in Rotarix recipients, including the infants who had PCV1 DNA detected in their stool. Finally, there was no evidence that infants with PCV1 detected in stool had an adverse event profile that differed from that in placebo recipients.

To close out the clinical part of the investigation strategy introduced by Dr. Hanon, the investigations were designed to address the question of whether the PCV1 signal present in Rotarix is associated with the presence of virus capable of causing infection in infants. As per our results, the PCV1 signal found in Rotarix was not associated with virus causing infection in humans, as determined by our testing.

In conclusion, as part of our investigations, we performed testing on clinical samples to evaluate if children who had been previously vaccinated with Rotarix developed evidence of PCV1 infection. The testing was done even though our expectation was that we would not find evidence of PCV1 infection. The testing was done even though our expectation was that we would not find evidence of PCV1 infection in children, since the virus is not capable to replicating productively in human cells.

We applied appropriate methodology to insure selection of the most relevant studies and samples and blinding of laboratory personnel. While our investigations revealed the expected pattern of PCV1 DNA detection in stool consistent with the transient passage of the PCV1 DNA present in Rotarix, the currently available data do not suggest occurrence of PCV1 infection in infants who received Rotarix in these clinical trials. Therefore, the results are consistent with the published literature, which indicates that PCV1 is not capable to causing infection in humans.

Finally, in light of the fact that the results I have just presented have only recently become available, we are now in the process of evaluating the feasibility and potential value of additional clinical testing.

I would now like to turn the podium over to Dr. Friedland, who will summarize the safety profile of Rotarix.

DR. STAPLETON: Actually, Dr. Dubin, I think there are going to be a few questions for you. I have one.

DR. DUBIN: Sure.

DR. STAPLETON: Do you have some data on the sensitivity of your antibody tests, and also on the timing of antibody responses to PCV1 in pigs?

DR. DUBIN: Yes. Maybe I can address the second question first. The timing of antibody responses in pigs who have been challenged has been assessed, and antibodies tend

to be detected relatively early following challenge. I believe within 14 days of infection, antibodies are detectable.

I would like to emphasize again that the way our serologic testing was designed, we assessed post vaccination antibody responses after completion of the full vaccination series, which was either two or three doses in Rotarix recipients.

Then I think the second question concerned the sensitivity of the assay. We were hoping Professor Nauwynck would be here, since he has run the assay and he developed the assay. But we believe the assay is actually quite sensitive in detecting the presence of PCV1. This assay is used to detect PCV1 infection in pigs, where sensitivity is relatively high. It has been adapted for humans.

One of the things to point out is that we have adapted the assay in a way that we think if anything, it would be skewed for higher sensitivity in humans. So an anti-human conjugant is used, even though the positive control is pig serum. So we believe this is a sensitive assay.

DR. LARUSSA: A couple of questions about the Q-PCR assay on stool. What was your positive control? What do you think the sensitivity of the assay is? And did you take some negative stool, spike it with virus and do a dilution

and see what you got?

DR. DUBIN: Yes. I might actually ask my colleague who has actually performed these assays to comment on some of those methodologic issues. I think the answer to the last question which I can provide is, yes, there were spiking experiments done as part of the controls. But I would like to introduce Jean Paul Cassart, who might be able to give some of the specific details about the PCR assay.

DR. CASSART: The sensitivity of the Q-PCR used for those two analyses is about 30 copies for PCR reaction. When you express that in terms of grams of stool, it is around four times 10_4 copies per gram of stool.

For the first question also, what we did is to spike negative stool with final container material, and it was able to recover two different levels of PCV DNA inputs and develop the Q-PCR reaction. So we spiked in order to recover 100 copies and ten_4 copies after spiking negative stool per Q-PCR reaction.

DR. GREENBERG: Just a couple of questions, and maybe a comment. It seems to me that the shedding on the early specimen is totally consistent with passive transmission through the GI tract, but it doesn't prove that. It is also consistent with short term replication. Either one could be an explanation.

Towards that end, quantitatively is the amount of

RNA on the day seven specimens lower than the amount you would have seen on the day three specimens? I realize you have very few here, but at least in theory it would look not good to me if the amount you found on the day seven was actually more than the amount you found on the day three. I am just curious about that.

The only other question is, you gave as evidence that this was unlikely infection that the lack of mutation found in this DNA genome of less than 2,000 bases in a couple of specimens. I don't think that is a valid assumption. We have a DNA virologist around here, but I would have to see how frequently you select mutations in pigs after a short term passage.

One last question. Did you look for rotavirus, quantitate the rotavirus shedding in the same specimens? Or had you had that data previously quantitatively?

DR. DUBIN: Let me answer the question first about the quantitation. Even though this is a Q-PCR, given the retrospective nature of the testing and the fact that the way stool samples were collected did not fully control volumes, we really don't feel that quantitation can be done. We used a quantitative result to determine whether the result is above the limit of detection or not.

That being said, with four samples positive, we do have numbers associated with this, but we only have one

positive sample per infant, so we could not assess patterns, because the four infants that were positive were positive at the earliest time point, and none were negative at the next time point.

DR. GREENBERG: I was only saying that your numbers are too small, but if there was way more on the two day sevens than on the two day threes, I understand it could have been diluted. But I just wondered whether you looked at that.

DR. DUBIN: With the very limited numbers we had, we did look at the data. We could not discern any real pattern, but we attributed that to the very small sample size.

Maybe to come back to the question about the DNA sequences, we have now done in these four infants genome sequencing not only for the fragments that were amplified by the Q-PCR reaction, but for a broader range of nucleotides across the genome. In two of the children we have the entire genome sequenced, and there is 100 percent sequence identity with the material in the final container. For the third infant 95 percent sequencing has now been completed, again 100 percent identity in the third child, 100 percent identity over 50 percent of the genome.

The real question is, what does that mean? Does that mean that -- is that evidence against replication? We

have speculated that since the virus has been adapted for Vero cells, and that is the source of origin of the virus in our manufacturing process, or the virus to replicate in human cells, which would be a different species, a cell line from a different species, it might need to adapt and nucleotide changes might be introduced. The fact that we don't see any nucleotide changes, we see an identical sequence, we take as supportive information for lack of replication, but we realize it is not conclusive.

Then the last question I think had to do with whether we quantitated or looked at rotavirus shedding in stools. In fact these studies were initially done to do just that. That is why we had these archived samples. We did not however do RNA detection in these samples. We looked at rotavirus antigen. We have found evidence, and this has been published, and it is also reported in our prescribing information, there is evidence of a detection of rotavirus antigen in stools in a sizeable proportion of infants for a limited period of time. It tends to be detected later, and it tends to persist for a longer period of time, which reflects, we think, the nature of the fact that the virus in the vaccine does cause limited replication in the gut, and that is part of the mechanism of action.

DR. GREENBERG: I was simply thinking that in some infants, your rotavirus may not have replicated all that

well. If you had a stool specimen -- so it is sort of like a marker for passage through the gut. So if you have an infant where the rotavirus isn't replicating well, and that stool specimen is negative for rotavirus and positive for the porcine virus, it would again be saying since the rotavirus might be there is greater quantities, it is like a marker for transit through the GI tract.

DR. HUGHES: To follow up on that question, do you know for things that you know don't replicate, let's just say DNA from something that an infant eats, what the expected transit time is?

If for example DNA from pork or meat or something clears much more rapidly, then I think you would have reason for concern.

DR. DUBIN: We have looked at the literature to see if there is any information about transit time through the infant gut for things that would be relevant. Unfortunately there really is not a lot of relevant information. What we have been able to discern is that the transit times in this infants in this young age range can actually be quite variable, and sometimes can be prolonged, and usually is shorter.

There is anecdotal information about passage of foreign bodies through the GI tract where transit times can be quite long, measured in days or even a week or more. So

limited information, but we believe that there is a range of transit times and we don't have any specific data looking at DNA markers, for example.

DR. COFFIN: Actually I have two points, one to that last point. A good marker for that would probably be looking for Vero cell DNA. There ought to be some specific mobile elements or something that one could easily distinguish that from human DNA. There is probably an awful lot of that in your vaccine and would probably be fairly easy to follow if you wanted to do such a study. I assume you haven't.

DR. DUBIN: We haven't done that.

DR. COFFIN: The other question relates to -- to get back to your point about non-replication. In two or three days or even in one or two weeks, even if this was HIV you would still see virtually identical sequences to the clonal infection you started with. I would not take the identity as meaning anything.

But I have a question as well. Was that a bulk sequence or a clonal sequence?

DR. DUBIN: This was a cloned sequence.

DR. COFFIN: The PCR product was cloned and then sequenced?

DR. DUBIN: I'll let my PCR colleague answer that.

DR. CASSART: What we did, we sequenced the

complete PCR product, not clones.

DR. COFFIN: So in that case you certainly wouldn't see any mutation. Any mutations that occurred during replication would have had to come up to be a substantial fraction of the population already. If you sequenced a bunch of clones, you might be able to see a little something, but certainly the bulk consensus would have remained the same, even for a highly variable virus like HIV. DNA viruses as a rule show much less variability on replication.

Finally, one more question on that. What is known about strain sequence variation from one isolate to another, from samples that might be in natural products or pork or different trypsin batches, even? What is known about genetic variation of this virus from one analysis to another?

DR. DUBIN: There are several published consensus sequences. The DNA sequence of the material identified in Rotarix has about 98 percent DNA sequence identity with the published sequences.

In terms of the level of variation in genome sequences for different isolates of PCV1, I don't know, Dr. Meng, if you would have any comment on that.

DR. MENG: It is about a two to five percent variations among all those sequences.

DR. DUBIN: Just one other point about the DNA sequencing. As I mentioned, our speculation has been that

because the PCV1 detected in Rotarix has been adapted for growth in Vero cell by changing the cell line -- but this is speculation, by changing the cell line, there may be nucleotide substitutions introduced.

We were actually trying to confirm that in a series of experiments where we are taking the output of virus from Hep2 cells, this is just the sequences, and seeing if there are any nucleotide substitutions, which we think would help interpret the data.

DR. ROMERO: A question and a comment. For your HIV positive patients, do you know what their CD4 counts or percentages were?

Second of all, the issue, going back to what Dr. Greenberg and others have alluded to, the fact that you are not seeing differences doesn't necessarily mean that replication is not occurring. Even for error prone replication like occurs in polio virus, you have got a much larger sequence. You are going to see a lot more changes over that. You have got a very small sequence here that you are dealing with relative to that size.

DR. DUBIN: The question about the level of immunocompromise in the HIV positive infants, these were relatively healthy HIV positive infants. We selected for the study infants that were not immunocompromised by any objective criteria. Nonetheless they were all HIV positive.

DR. DEBOLD: I can appreciate that you were working with samples that were collected in the past, so you are stuck with the specimens that you have. But how do you know that the virus actually cleared? The absence of virus at day seven, 15, what have you, in blood and stool, does that necessarily mean that the virus cleared the body? Couldn't it be somewhere else, like lymphoid tissue or some other place?

DR. DUBIN: In order for the virus to actually infect other tissues, tissues other than gut cells, we would expect that it would need to actively replicate in the gut, and we think that it would very likely in that situation induce an immune response.

So we think the serologic data are important, because after two or three doses of Rotarix, even in children that had DNA detected, we show no evidence of seropositivity, which is consistent with the serologic evaluations that have been previously published, in individuals that are heavily exposed to contaminated pork products.

DR. HOWE: I just wanted to add to that, to say that also, as you saw the number of samples that we tested were relatively limited, we feel that the finding there is reinforced by the literature and vice versa, that this is what was expected to be the case and the outcome, based on the literature and what we know about how the virus behaves.

I also did want to respond to the one question that was asked earlier about whether or not stool testing was done in the SCID case. It is not part of the clinical investigation proper. The subacute combined immunodeficiency case came to our attention through spontaneous reporting. Therefore, we don't have ready access to the stool sample, but we have requested it, and we are going to come to test it.

DR. STAPLETON: Dr. LaRussa, you had another question?

DR. LARUSSA: Yes, just one last point. Maybe we will hear this later, but what happens in the pig when you put PCV1 and you look in the stool a week later? Do you see any sequence diversity?

DR. DUBIN: Apparently this exact experiment hasn't been done. I know Professor Meng has done some challenge studies with PCV1, but I think in most of the challenge studies the route of inoculation was not intraoral, it was either systemic or intranasal, if I am correct, intramuscular. And PCV2 has been given intranasally, but those studies haven't been done.

DR. STAPLETON: We will move on now.

DR. FRIEDLAND: Good morning, Mr. Chairman and members of the committee. I am Dr. Leonard Friedland from the GSK Clinical and Medical Affairs Team.

We have heard throughout this morning about the biology of PCV1, and that it has been in Rotarix from the early stages of development, throughout the clinical trials and postmarketing until today. Our primary focus is always on patient safety, so in parallel with GSK manufacturing and clinical investigations, when we first learned about the presence of PCV1, we also critically reviewed the scientific literature and consulted experts. The unanimous conclusion from all sources is that PCV1 is not known to be infectious in humans, nor does it cause disease in humans or any other animal.

The Rotarix safety database is large, robust and extensive. It is continuously monitored over time and consistently demonstrates the safety of Rotarix.

While there is no specific PCV1 lens with which to query our database, because in the absence of disease there are no symptoms, what I can do today is the following: Provide an overview of vaccine efficacy and safety from our large clinical trial database, which supported U.S. approval in 2008, our worldwide postmarketing safety and effectiveness experience, and favorable benefit-risk in the presence of PCV1.

Rotarix has had one of the largest vaccine development programs. Supporting U.S. licensure were 11 studies with more than 75,000 infants enrolled, 40,000 of

whom received Rotarix. Efficacy was evaluated through two years or two Rotarix seasons after vaccination, so therefore safety was also evaluated in many of the infants for up to two years after vaccination. The development program included a large safety study in more than 60,000 infants, specifically powered to assess for intussusception.

The efficacy data come from more than 24,000 infants randomized in two placebo controlled studies conducted in 17 countries. Rotarix was shown to prevent all severities of rotavirus, including severe rotavirus, gastroenteritis, rotavirus hospitalizations and medically attended visits, as well as protect against commonly circulating rotavirus types. Clinical trial data collected since U.S. approval continued to demonstrate efficacy in various populations, including Asia and Sub Saharan Africa.

The 11 clinical trials which supported U.S. licensure were conducted between 2000 and 2007. Analyses over time demonstrate a consistent safety profile. The safety profile of Rotarix is similar to placebo. This has been demonstrated in an integrated summary of safety of eight clinical trials involving more than 70,000 infants; roughly half received Rotarix.

Infants were monitored for serious adverse events and specific solicited adverse events. The incidence of serious adverse events and solicited adverse events occurred

at similar rates in Rotarix and placebo subjects. Importantly, no increased risk of intussusception was found compared to placebo.

Now that I have reviewed the database that supported licensure, I will focus on our large global postmarketing experience.

Pharmacovigilence activities are in place. GSK has a worldwide network of safety personnel who analyze adverse events and expedite reporting for worldwide regulatory agencies. For spontaneously reported intussusception cases, enhanced pharmacovigilance is used, comparing the number of cases observed to the number expected.

Since the worldwide launch in 2006, over 69 million doses of Rotarix have been distributed, including 2.5 million in the United States. The company has received approximately 3,000 adverse event reports, appropriate 1200 of which are considered to be serious as defined by regulatory criteria. This represents a reporting rate of 4.3 per 100,000 doses distributed. This rate is consistent with reporting rates expected with new vaccines.

This table lists the postmarketing events most frequently reported as occurring after Rotarix administration above a reporting threshold of .5 per 100,000 doses. It is of interest to note that in the clinical trials, the incidence of diarrhea, vomiting and fever occurred at similar

rates in Rotarix and placebo subjects.

To see intussusception on this list is not surprising, given the large awareness of this event following the Rotashield experience. To asses whether the number of intussusception reports received reflects the natural background rate of intussusception in the countries where the reports originated or potentially reflects an increased risk following Rotarix, observed versus expected analyses have been conducted. The number of cases reported to the company on a worldwide basis does not exceed the number expected to occur by coincidence after vaccination.

We searched our postmarketing safety database for all reported fatalities since worldwide approval in 2004 through March of 2010. In the U.S., a total of six deaths reported in temporal association with Rotarix. In each case other routinely recommended infant vaccines were concomitantly administered. SIDS, cardiopulmonary arrest, status epilepticus, traumatic brain injury and fatal airway obstruction were the causes of deaths. None of the reports suggested a causal connection to Rotarix.

Since the U.S. market introduction and prior to the identification of PCV1 in Rotarix, three events were added to the postmarketing section of the prescribing information. The three events are intussusception including death and temporal association, Kawasaki disease and rotavirus

gastroenteritis in patients with severe combined immunodeficiency syndrome. These events are also listed in the prescribing information of the other U.S. licensed rotavirus vaccine.

Various phase IV clinical trials have been conducted, including safety and immunogenicity trials in HIV positive infants and in premature infants. Rotarix was found to be immunogenic and well tolerated in these particularly vulnerable populations.

We have also conducted a transmission study between twins, demonstrating low rates of transmission with no associated gastroenteritis symptoms.

We are also conducting a number of observational studies worldwide to further monitor the safety and effectiveness of Rotarix. One ongoing study in the United States will include 55,900 infants receiving Rotarix to assess the risk of intussusception and other serious adverse events. Vaccine effectiveness studies are also being conducted.

The published studies shown here demonstrate the considerable impact and effectiveness of rotavirus in preventing severe rotavirus disease in real life settings. These data together demonstrate that Rotarix is a very effective tool for significantly reducing morbidity and mortality due to rotavirus gastroenteritis.

To summarize and conclude my presentation, rotavirus infection is the leading cause of severe diarrhea in both developed and developing countries. Prior to the development of vaccines against rotavirus, worldwide one child died from rotavirus every minute. To date, vaccination is the only effective preventative strategy. Its widespread use has the potential to prevent about two million deaths over the next decade.

To date, Rotarix confirms robust protection against rotavirus gastroenteritis. As acknowledged by the FDA at the time they notified health care providers of the presence of PCV1 in Rotarix, Rotarix has been extensively studied before and after approval and found to have an excellent safety record.

Material from PCV1 has been present since the initial stages of the vaccine's development, throughout clinical trials and postmarketing until today. Thus, all of the safety data I have reviewed reflect exposure to PCV1 supporting the safety profile of Rotarix. So overall, the benefit-risk for Rotarix remains favorable.

I will now hand the podium over to my colleague, Dr. Howe, unless Dr. Stapleton would like to entertain questions.

DR. STAPLETON: I think it is useful. I know we are running over, but it is nice at the time this would feed

into our discussion. So are there questions?

DR. WHARTON: Thank you for that presentation. Is there any information from the post licensure safety experience about Kawasaki disease?

DR. FRIEDLAND: Yes, thank you. We have one report in our postmarketing spontaneous reports of Kawasaki disease since Rotarix had been licensed in the U.S.

DR. DESTEFANO: I have a question about the long term safety assessment, first of all from the preclinical data. In any of those studies, what was the long term follow-up for any of those studies, and what have been the findings from the longer term follow-up?

DR. FRIEDLAND: Actually, four of the clinical studies that evaluated efficacy were conducted for two years, looking at efficacy. Those four studies were part of the licensing application. When we looked at serious adverse events and other adverse events reported in those four two-year follow-up studies, there were no differences in reporting rates between placebo and Rotarix recipients.

In addition, since U.S. approval we have conducted and finished a three-year study of efficacy in Asia, and we have collected and looked at serious adverse events through the three years of follow-up. Approximately 8400 subjects have been followed out for three years, and there are no differences in reporting rates of serious adverse events

through three years of follow-up in those 8400 subjects.

DR. DESTEFANO: Similarly, do you have any postlicensure monitoring data for longer term follow-up?

DR. FRIEDLAND: The postlicensure monitoring that we are doing is, number one, through spontaneous reporting. Of long term follow-up, we have two ongoing observational studies, in particular to mention one study being conducted in Mexico, where we looked consecutively at birth cohorts in a self controlled study, where it is an event driven study looking for a specific number of intussusception cases. There are other adverse events that can be reported in that study, and we do monitor those as they come in.

In our U.S. observational study which will enroll 55,000 infants, as of June of 2009 about 9300 infants have received Rotarix in that study, and similarly 50,000 control subjects. While the data are not unblinded, we don't see any raw numbers of adverse events that would raise any concern.

DR. TSAI: I just wondered whether you had considered doing any kind of proportionality analysis. I know the value of that is somewhat controversial. Maybe others could comment, but since you didn't have a lens, as you put it, to look at specific adverse events, perhaps that would have been a way to look at signals.

DR. FRIEDLAND: Yes, thank you. We of course have been thinking along the same lines. We have looked at

disproportionality analyses through our postmarketing database. We see no evidence of disproportionality in subjects in our database who have received Rotarix vaccine compared to other vaccines in our database.

DR. STAPLETON: Let's move ahead then. Dr. Howe.

DR. HOWE: Thank you. I am just going to take a few minutes to summarize what you have heard from GSK and other presenters today, and to conclude our presentation, and then to share with you GSK's next steps.

We heard from Dr. Meng that PCV1 is a clinically benign virus which is commonly found in a number of products. It is not known to cause any illness in humans or animals. However, now that we know this is an advantageous agent, no matter how benign, is in our product, GSK is committed to manufacturing using PCV1-free manufacturing materials.

Developing a new manufacturing process is a complex undertaking, however, and it will take time. So the question is, what do we do in the immediate future.

For this, we turn to GSK's investigation as well as the benefit-risk assessment on Rotarix containing PCV1. As we heard from Dr. Hanon, in permissive cells, low levels of infective virus are present in the Rotarix production process, and infective viral particles cannot be excluded in the final container in an amount which is below the limit of detection. While the virus is capable of replicating on

permissive cells, at this stage the evidence does not indicate productive infection in human cells.

Importantly, current available data from our clinical investigation do not suggest the occurrence of PCV1 infection in infants who receive Rotarix in clinical trials. All of these findings are consistent with what we know from the literature as presented by Dr. Meng.

In addition, there is an extensive body of evidence, both before and after approval, supporting the safety of Rotarix containing PCV1. As you just heard from Dr. Friedland, this includes data from tens of thousands of subjects in clinical trials as well as postlicensure experience with millions of doses worldwide. All available evidence supports the safety of Rotarix containing material with PCV1.

We should also consider the recognized substantial benefit of vaccination against rotaviral disease, both in the developing world and in developed countries. Studies in the U.S. have shown that vaccination has resulted in 60 percent reduction in rotavirus disease as compared to the pre-vaccine era. You also heard it is estimated that expanded widespread use of rotavirus vaccines globally could prevent approximately two million deaths over the next decade.

So taking all of this evidence in our assessment into account, the Rotarix benefit-risk remains unchanged by

our new knowledge concerning PCV1. What I mean by that is that the benefit-risk profile remains favorable.

Based on this favorable benefit-risk, here are GSK's proposed next steps. First of all, we will continue the investigations, including ongoing manufacturing investigations and also an evaluation of the feasibility and potential value of additional testing. As I just said, we are committed to implementing manufacturing changes using PCV1-free starting materials. But again, this is a complex process, and it will take time.

Meanwhile, of course we will continue comprehensive pharmacovigilance activities that are already in place worldwide.

In summary, based on the favorable benefit-risk and the science reviewed today, GSK is already continuing to make Rotarix available worldwide while manufacturing changes are being evaluated and implemented. Should the committee and the FDA deem it appropriate, GSK is also prepared to continue to make Rotarix available in the U.S., in which case we would propose to update the registration file and also the label to reflect the presence of PCV1. This will be in the interest of transparency and also to maintain regulatory compliance.

Finally, as a global manufacturer, GSK remains committed to developing and manufacturing high quality, safe and effective vaccines to address important public health

needs throughout the world. Today the global vaccine community faces new challenges, and as new technologies continue to emerge, more challenges will certainly follow. The rotavirus vaccines are really just one example.

GSK believes that decision making must be based on sound science and an appropriate benefit-risk algorithm on a case by case basis whenever new findings are detected. These are complex and difficult issues for which we are grateful to receive feedback from this VRBPAC, from the FDA and from regulators worldwide. We will work with FDA and others to overcome the challenges in a manner that best addresses global public health needs through the continued supply of much-needed vaccines.

Thank you very much for your attention.

DR. STAPLETON: Thank you, Dr. Howe. I'm sure that many people would like to ask questions, but I think for the sake of time, what we will do -- and I apologize to Dr. Allan -- we will move his talk until after the break, and then during our discussion we can ask questions of Dr. Howe and GSK.

So we will take a break for 15 minutes.

(Brief recess.)

DR. STAPLETON: I would like to call the meeting back to order after the break, and ask Dr. Gordon Allan from Queen's University-Belfast to give us an overview on porcine

circoviruses.

Agenda Item: Porcine Circovirus: An Overview

DR. ALLAN: Thank you, Mr. Chairman, and good morning, ladies and gentlemen. First of all, I want to thank the FDA for inviting me to Washington to talk to you about porcine circovirus. My initial brief came from Philip to give a talk and provide an overview of PCV1, its pathogenesis, its protection, ability to infect other species, inactivation, et cetera. My brief was changed about two days ago to include PCV2. My time was not changed, so I put in a few slides on PCV2, which for me is much more interesting than PCV1. I'll give you a few slides on PCV2, but essentially I am going to talk mostly about PCV1.

Taxonomy. What is PCV1 and where does this fit in the family of circoviruses? PCV1 is a virus with circular, single-stranded DNA genomes. There are a number of bacterial and plant viruses, and then we have family *Circoviridae*, in which there are currently two genera, genus circovirus which contains PCV1, beak and feather disease, PCV2, pigeon circovirus, goose circovirus, there is a canary circovirus as well, and there are a couple of others. Genus gyrovirus, which is chicken anemia virus. Then this is genus *Anellovirus*, which contain the TT viruses, essentially.

You will be pleased to know that this doesn't actually mean anything. It is probably going to be changed

next year, and they are going to reclassify everything yet again. But that is the current status, anyway.

Circovirus, I suppose you could say, started in 1974. There was very little done between 1974 and 1994 on circoviruses. Psittacine beak and feather disease was probably the one that most people worked on, discovered by Brian Ritchie. Brian came to my lab in Belfast and we worked together on the three circoviruses that were known at that time.

Chicken anemia virus, a virus infecting chickens, horizontal transmission, vertical transmission. You only get disease with vertical transmission with chicken anemia virus. It causes severe anemia in chickens, as the name says. And of course porcine circovirus, Tischer et al., 1982.

This is a sick parrot, as you can probably tell. This parrot has got beak and feather disease, a very nasty chronic wasting disease of parrots, which eventually die. This is a circovirus. This is the picture produced by Tischer way back in 1984, I think it was.

Tischer et al. did most of the work on porcine circovirus. They first encountered it in 1974, when they published a paper which was a characterization of a picoma-like virus contaminating PK-15 cells. It has a density of 1.37, the cesium chloride, and they reported it as having an RNA genome in that paper, interestingly enough.

Then nothing happened. Everybody said, that is very interesting, et cetera, and nobody did any work on it, except Tischer, who eight years later published a second paper. This time, the picoma virus was growing in pig cell lines. It was dependent on S-phase. In other words, it was actively dividing cells to replicate. The big break-through. They reported a single-stranded circular DNA genome, and they named the virus porcine circovirus, very logical.

Tischer then came up with limited serological studies in Germany, and there were other studies in the U.K. We did some studies showing that this circovirus, now called porcine circovirus 1, was widespread in swine throughout the world.

This is quite interesting. In '82, the same group in Germany failed to demonstrate antibodies in rabbits, mice, cows and humans, and they also failed to show any clinical disease associated with PCV1 in mini-pigs.

Essentially this is where we were at the end of 1989, beginning of the 1990s. We had three viruses, chicken anemia, Circo, psittacine beak and feather. We knew the size, we knew the density, we knew the genome structure, we knew the nucleotides on the genome and we knew the polypeptides, and that was that, really. There wasn't very much being done on circovirus.

Then I was tasked with doing a Ph.D thesis, which I started in 1989. It took me a long time, as you can see, but I got there in the end. We started working on porcine circovirus 1 as it was then, the PK-15 contaminant. What I am going to do is run through some of the results that we published on my thesis, and then compare them with other results that have been generated to date.

Bear in mind, the work we did on -- this is quite important -- the work we did on porcine circovirus in those days was on the PK-15 contaminant, not a fetal isolate of PCV1. The PK-15 contaminant has been passed through PK-15 cells forever. It probably doesn't really represent a fetal isolate of PCV1.

The first thing which is good Ph.D fodder is to look at what cell lines the virus grew in. We took a selection of the cell lines we had in the laboratory, chicken cells, continuous porcine kidney cells, primary pig kidney, primary testes, bovine cells, HeLa cells. I highlighted these two in red, because as it turned out, these were the two important cell lines.

The virus grew initially in pig kidney, pig testes, pig lung. It didn't grow in chicken cells, surprise, surprise; it is not a chicken virus. It did grow in bovine kidney cells, which was quite interesting, and infected bovine kidney cells. But *en passage* the number of cells

decreased, and eventually there was no replication.

However, if you look at the continuous cell lines we used, this is another continuous pig kidney, we call it Hanover because we got it from Hanover, and it was a PCV1 free cell line. Vero and monkey kidney. The virus grew and replicated best in PK-15, the host, and also in Vero, which was surprising, actually.

If you *passage* it through PK-15 and you *passage* it through Vero, you find that it grows as well if not better in Vero than it does in the PK-15 cell line, which in retrospect if you were choosing a cell line not to grow a PCV1 contaminant vaccine in, it would be Vero. But hindsight is a wonderful thing.

This just gives you a summary of that. This is a picture of primary infection in Vero with PCV1 as it was then. You get a lot of small speckled fluorescent cells. This is stained using monoclonal antibodies. If you *passage* it through Vero, the virus grows and you eventually get a CP in Vero if you *passage* it long enough.

Primary pig kidney was an homologous cell line, if you want to use that terminology; pass it through that and you get the same picture. There is very little difference. In fact, I would argue you get more virus produced in Vero than you do in PK-15.

Interestingly as well, we find that when you pass

this virus through Vero, it changed antigenically, as determined with its reactivity with a panel of monoclonal antibodies reproduced. I'll show you some of those results later.

This just shows you what happens. These are duplicate cell cultures. This is stained with a monoclonal antibody 2E1, which stains the viral *passage* to PCV1, and this is stained with a monoclonal antibody 1H4, which does stain the PK-15 *passaged* PCV1, but doesn't react at all with the viral *passaged* PCV1.

So as you pass it through Vero, it changes antigenically. What we did not do because we weren't doing those things in those days, we did not sequence any of these viruses. But I think they are probably still there, and if anybody wants to sequence them, they are welcome. I'm not going to do it, but I think they are still there.

The one thing I would question, we experimentally infected pigs with the PK-15 isolate. We did not experimentally infect pigs with the PK-15 Vero isolate. In retrospect it would have been interesting to do.

Epidemiology. As X.J. Meng said, widespread in pigs, the virus is widespread in pigs. We did not detect any antibody in sheep, cattle, turkeys, chickens, humans or goats in our study. PCV2-1 antibodies have been about for decades. We went back as far as 1969, and we found large numbers of

positive pigs in 1969.

Seroconversion occurs, certainly in Northern Ireland. It comes quite late. Seroconversion occurs between 12 and 13 weeks of age, which is quite late for a normal pig virus.

A very simple table, self explanatory. There is the species. That is the number of sera tested. Widespread in pigs, nothing. We tested it at a 1/40 dilution.

We have this paper from Tischer which came out in 1995, which is quoted in the literature every time you lift a paper on serology for PCV2. This paper is quoted as having detected antibodies in any number of species.

Humans, mice and cattle; to date I don't think there are any other reports. I personally, this is a personal opinion, I don't believe these guys demonstrated antibodies in humans.

This is a plate of the picture from the paper. This is rabbit hyperimmune PCV1 antibody they produced, immunostaining. This is a monoclonal, and this is swine immune sera. These are all done in a 1/10 dilution.

Anybody doing immunofluorescence at a 1/10 dilution is asking for problems. Why they would want to do it, I don't know, but a 1/10 dilution is a nightmare if you are looking at fetal sera. From any species you get a lot of non-specific staining.

Personally, I believe that this is non-specific staining. I don't believe this has anything to do with PCV1 in pig, and I don't think it has anything to do with porcine circovirus in humans cross reacting either. I just think it is non-specific staining.

I'll say no more. I just think it is non-specific staining.

I mentioned monoclonal antibodies. We produced a panel of monoclonal antibodies specific to PCV1. We also have a wide range of monoclonal antibodies specific to PCV2. These were used to differentiate. When we isolated PCV2, we used the monoclonal antibodies to differentiate between 1 and 2, and that formed part of the basis of the patent.

We used them to develop an ELISA. We used them for physico-chemistry. This is probably the most important one: we used them to differentiate PCV1 strains, in inverted commas, to look at the PCV1 Vero. Towards the end of this thesis, in the last six months of this thesis, we isolated two PCV1 Vero isolates, and we used them to differentiate those as well, because the monoclonal antibodies had a different staining pattern in those isolates compared to the pig kidney.

This is a characterization of the monoclonals. They are all IgG's of some sort. One of them is neutralizing, this one here, at 1/40, and the rest were not.

This is a complicated slide, I don't want you to go

into it in any detail, but suffice to say there were a number of staining patterns with these monoclonals which are described here. The reason I show you this is, those three monoclonals, including the neutralizing one, did not react with PCV1 that was *passaged* through Vero. It lost reactivity. That 2B7 there also lost reactivity to the PCV virus that was passed through Vero.

We also used it to differentiate fetalACE. This is one of our fetal ACE's from PCV, from an abortion, stillborn. The PCV1 stained with 7B4, and we got this reactivity. The same monoclonal staining the PK-15 virus, there was a different pattern of reactivity, so we did use that to differentiate our fetal isolates.

Again, good Ph.D fodder, some biological and physico-chemical properties. What we did was purify PCV1 out of cell cultures using Triton X 114 to extract it. For anybody who wants to extract PCV1, use Triton X 114; it is much more efficient than any other detergent. We did purify through sucrose and cesium chloride using our monoclonal antibodies captured ELISA.

The usual stuff here, pH3, chloroform 5670, resistant to pH3, resistant to chloroform, resistant to 70 degrees.

There is a paper in the literature that says PCV2 is inactivated at 90 degrees for five minutes. For PCV2 we

knew that formalin and BPL will inactivate it, and we know that PCV2 and PCV1 is found in pig's meat. The physico-chemical properties of PCV1 are in my opinion going to be the same as PCV2.

Buoyant density. Again we confirmed Tischer at 1.36 and 1.37. Sedimentation coefficient was 57S.

Pathogenesis. We did two pathogenesis studies or two different methodologies. We looked at in vitro studies and cultures of peripheral blood mononuclear cells, bone marrow, lymph node, alveolar macrophages, and we also did cultures from swine, sheep, cattle and humans.

What we found essentially was that PCV when replicated in porcine monocyte macrophages in vitro, PCV1 did not replicate. I personally could not get PCV1 into human macrophages as derived from peripheral blood mononuclear cells. I could get it into bovine macrophages, I could get it into sheep macrophages or monocytes, but I couldn't get it into human cells by simple inoculation.

We looked at the immune functions of the cells once they were infected, and there was very little change. There was no effect on phagocytosis. There was a slight effect on the expression of Fc receptors, and there was a very transient upregulation of MHC class two, but essentially there was very little immunomodulation in vitro.

This is just a table that shows you what happens

when you infect the mononuclear cells from porcine cultures and bovine cultures. The virus goes into both. The virus is degraded, and then we start getting new infectious virus coming out. In the bovine cultures we got no new infectious virus coming out, so the virus in our opinion was replicating in porcine peripheral blood mononuclear cells but not in bovine.

Pathogenesis, part two, experimental infection of CV in pigs. These are colostrum deprived pigs. They are not Caesarean derived, they are not CD/CD and they are not gnotobiotic pigs. They are snatch farrowed colostrum deprived pigs. They are not biologically clean. They are in a clean but not in a sterile environment, and they are exposed to a number of infectious agents, unknown presumably.

We infected these by the oro-nasal route. What I mean by the oro-nasal route. Somebody was mentioning nasal inoculation. If you infect a pig intranasally, which we tried to do, you will find the pig will swallow over 25 to 30 percent of the inoculum; it happens. So if you are inoculating a pig oro-nasally by dropping it into its nose, the pig will end up swallowing over 25 percent of that inoculum, so you are effectively inoculating them oro-nasally.

Controls were the usual mock-infected with PCV-free. These are our pigs. They are held in a clean but not

sterile environment. They are fed milk and they are raised by my colleagues.

A table of where we picked out the virus after 11 days. Lymph nodes, thymus. This is by indirect immunofluorescence. Lung, spleen. Buffy coat, we got one there. We got some in the small intestine, but in general we did not get a virus spread. We got no clinical disease.

There is a caveat on that. In retrospect, this experiment was extremely flawed, because we know how that if we infect the same pigs with PCV2, you get no clinical disease at 11 days. You get clinical disease starting about 15, maybe 16, maybe 20 days, and the animals will eventually die about 25 to 30 days. So by 11 days post infection, we shouldn't have expected -- we didn't know then, but we should not have expected a clinical disease.

I'm not saying there would have been clinical disease. I'm saying that in retrospect we should have left the pigs longer.

The second caveat is that we know also that if we infect these type of pigs with PCV2 alone, we don't get any reasonable disease. If we infect these pigs with PCV2 alone and immuno stimulate them or co-infect them, then we get massive disease. None of this was done to these pigs, so that is the caveat.

I'm not saying we would have had clinical disease

if we had done all this. All I am saying is, in retrospect we should have done it and we haven't done it.

Virus distribution. We got quite a lot of virus, PCV1, thymus. It was one of our better cells, probably dendritic cells and thymus. This is lung, fluorescence, PCV1 in lung. This is spleen with PCV1. So there was a fair distribution of virus as determined by indirect immunofluorescence.

We looked at field material. We looked at fetal sera. We checked them for antibody, they were all negative, but we did eventually isolate two PCV1 fetal isolates from fetal material, from fetal spleen samples and from pooled samples, and those viruses were not laboratory contaminants. We made sure. The first thing my boss said to me is, that is a laboratory contaminant, you have got the PK-15 virus in there. I said, no, we haven't.

We didn't do sequencing in those days, but what we did was, we screened them with a panel of monoclonal antibodies, and the reactivity patterns were different.

Porcine circovirus 1 conclusions. Actually Dr. Meng has gone through most of this. Small icosahedral single stranded. No evidence of infection of species other than swine with PCV1, and no reports of disease in swine associated with PCV1 infection. That is a statement of fact. PCV1 DNA can be infectious, but you have got to make a clone

with a tandem repeat on it.

Since the discovery of PCV2 in 1996, virtually nobody has done any work on PCV1. PCV1 was very interesting and was a great molecular tool for molecular biologists who wanted to play around and look at things with genes, et cetera, but in the real world PCV1 was not a problem then. All of a sudden PCV2 popped up, which was associated with disease, and everybody dropped 1 and started working on 2.

Some work has been done. There are a few molecular biologists on it. Annette Mankertz did some work on genomic organization. What I would like to do if I was still in the business of PCV, which I am not essentially, is go back and do some work in the PCV Vero isolate we have and also the two fetal isolates. As you know, as X.J. mentioned, PCV1 contamination has been detected in vaccines.

Philip asked me to mention something about detection methodologies, but I'm sure that will be covered later. I am just going to give you the four or five detection methodologies you can use.

Differential PCR has been covered extensively by previous speakers, so I'm not going to do that. You can use any metagenomics processes, which I don't understand, but I'm sure Philip will explain those to us later. You can grow PCV in cell culture, but we know that is a relatively insensitive technique. You can use antigen capture ELISA, again not very

sensitive. You can use electron microscopy, extremely not sensitive, or you can use experimental infection of CD pigs, our type of pigs, but then you have problems, sometimes we get contaminations. Or you can use experimental infections of gnotobiotic pigs, which is probably the ultimate sensitivity.

We know from work we have done in conjunction with Morris, Panzer and Hanz that if you titrate PCV2 in cell cultures and use the dilution to infect pigs, you will find there is probably a three to four log difference in the infectivity titer in pigs as opposed to cell cultures. So the most sensitive methodology to look for infectious material is to titrate in a clean pig system.

There are monoclonals, polyclonals, virus pools; all the equipment and materials you need to do this are available.

So that is PCV1, essentially.

Quickly, PCV2. Philip asked me to do something on that. First recognized in '97. First seen as a disease in 1991. PCV2 is not a new virus, it is a newly recognized virus. It is a necessary causal agent of PMWS, which is a very devastating disease; I'll show you some pictures of it later.

The genome is 75 percent homologous with PCV1, and the ORF-1 85 percent, and the ORF-2 62 percent.

PMWS, postweaning multi systemic wasting syndrome, is what it describes it to be. It is a syndrome of pigs that initially was seen in post wean pigs where they wasted and eventually died. When it started in Eastern Canada and Northern France and spread to Europe, you were looking at deaths in the region of 40 percent of pigs. If you have a thousand sty unit producing 10,000 piglets twice a year, a 40 percent death rate is quite high. So it was a very severe lethal disease in pigs. It is now controlled by vaccination.

There are at the moment three distinct genome groups of PCV2. There are two perhaps being recognized in China, which I have heard about but haven't read about. Some people say the virus appears to be changing. I'm not quite sure whether I agree with that. I see Hans is here; he will probably argue that it is. Hans will argue the virus is changing, and it is becoming more virulent. I don't know whether I agree with that or not, but we will see.

I would perhaps argue and did argue for some considerable time that the virus is a novovirus, but the way we raised pigs and the way we bred pigs changed dramatically in the time scale when this disease started to emerge. The pig we rear now is totally different from the pig we reared in the 1930s, '40s, '50s, '60s, '70s, et cetera. It is a totally different animal.

There appears to be natural recombination of PCV1,

PCV2 in vivo. The caveat to that is, it is not a natural recombination. That paper was published, and there is a logical explanation for that finding, which is not to say it is a natural recombinant. You can ask me if you want, but I don't want to say anything more about it at the moment.

Common clinical signs of PMWS. These are pigs, obviously. These are all litter mates. This animal has got wasting disease. You can see it is about half the size of this animal. This is a severely wasted disease animal. This animal died the next day from PMWS.

You get very enlarged lymph nodes. You get enzootic pneumonia, you get very enlarged mottled kidneys, hugely enlarged lymph nodes. If you do immunocytic chemistry with PCV2 antibodies you get massive amounts of PCV2 antigen associated with the lesions in these pigs. So PCV2 is the cause of the disease.

That is all I have time to say. I could talk for two hours on PCV2, but that is all the time I have to say.

Philip, I am blaming you for a lot of things here, but you asked me to mention other swine viruses that might become important in the future, so I am going to mention them.

There are four other newly emerging swine viruses that in retrospect as what has happened with this vaccine, may or may not become important in the future with other

human vaccines, where pig products are used to produce them.

Porcine bocaviruses. There are human equivalents of bocaviruses, but there are porcine bocaviruses. These are members of the parvovirus family, essentially. We in Belfast have two new isolates of porcine bocaviruses growing in cell culture. This is one of them. We have monoclonal antibodies, et cetera.

The Swedes, Sandor Belak's group with our cooperation, has also got a partial sequence now from a bocavirus in Sweden, which is different from ours. I believe there is a sequence now being published in the U.S. from a bocavirus in the U.S., which again is different from ours and from the Swedish one. So swine bocaviruses are emerging, again that could be a problem if we continue to use pig trypsin.

Porcine parvovirus 2. This has been around for quite a long while, 2001. Very little work has been done on it. We looked at pigs in Northern Ireland, and we found 40 percent positivity by PCR. We have molecular diagnostics for that.

Porcine hokovirus, isolated from swine in Hong Kong. We have about 9.2 percent positivity in Northern Ireland, and again we have molecular diagnostics available for those as well.

Probably the most important one of these which is

not a member of the parvovirus group is currently a member of the circovirus group, is the swine TT virus or swine Torque Teno virus. Two genome types at the moment. There are human equivalents of these viruses, and this is probably the most important. I am not an expert on swine TT. Steve Krakowka, who is out there somewhere, is. If you want to talk to him about this, you can. There is a high probability that recombination events between human TT's and swine TT's will and can take place.

Vertically transmitted in swine. We have shown that some of the veterinary vaccines are certainly contaminated with swine TT. We have molecular tools again to look at swine TT, and bioassays are available in gnotobiotics in Steve's laboratory.

That is me, finished essentially. I want to thank the people on that list. I want to thank the FDA for their invitation again. I especially want to thank Denise, because she put up with a lot of hassle and e-mails from me during the organization.

Thank you.

DR. STAPLETON: Thank you, Dr. Allan. I'm sure there are a few questions from the committee.

DR. LARUSSA: I want to follow up on one comment you made. If I understood what you said correctly, PCV1 changes antigenically when it is passaged in Vero cells and

monoclonal antibodies react differentially to whether the virus, right?

DR. ALLAN: Yes.

DR. LARUSSA: The comment about what is the substrate for the antibody assays that are being used to look for infectivity in human, if you are using for lack of a better term, the wild type or the PK-15 virus as your substrate, would you not pick up antibody to the Vero cell adapted virus?

DR. ALLAN: The answer to that is that I don't know, but I suspect Hans will know. You are using the PK-15 contaminant as your substrate, so you are using the cell contaminant. Do we know if it is anything like wild type PCV1?

The question is, is the PCV1 substrate and the ELISA close to, near from, similar to, the PCV1 Vero.

DR. NAUWYNCK: What I am using is not an ELISA, but an immunotrax(?) and monolayer assay, if you are talking about detection of antibodies, serological testing. So I am using as the basis cells inoculated with -- it is just PK-15?

DR. LARUSSA: Yes.

DR. NAUWYNCK: PCV1?

DR. LARUSSA: Yes.

DR. NAUWYNCK: That is the target, that is the antigen. Then we add the human polyclonal sera, and then we

detect if there is reactivity, yes or no.

DR. LARUSSA: But you don't use the vaccine circovirus.

DR. ALLAN: You don't use the actual circovirus.

DR. NAUWYNCK: I don't.

DR. LARUSSA: What I am saying is if humans are being exposed to the Vero cell adapted virus, and that change is antigenically different than the PK-15 adapted virus, if you are looking for human antibody or lack of antibody as an expression of infection, then the substrate has to be the human adapted virus and not the PK adapted virus.

DR. NAUWYNCK: There is sufficient cross reactivity because your monoclonals are not doing the job. The polyclonals will do it. Now if you use the PK-15 within that, the PCV1, if I use a polygonal because we don't have humans here, but if we use our pig polyclonal antisera, if we use that one for PCV1, PK-15 PCV1, we detect it. If we use the cells infected with vaccine virus, we still detect it. So the polyclonal, you will still detect it.

DR. LARUSSA: I'm not talking about detection of virus. I am talking about detection of antibody in human sera. So if the antibody in human sera is going to be a reaction to the virus that the humans were exposed to, which is the Vero cell adapted virus, if you use as your substrate the PK-15 adapted virus, the humans may not make antibodies

to that, and you will say they are not infected. But they may be infected; you are just not using the right substrate.

DR. NAUWYNCK: I think, Gordon, you should tell that there are still a lot of monoclonals that are reactive. There are one or two that do not react.

DR. ALLAN: There were nine monoclonals which we made it to PCV1 PK-15 contaminant, and with a number of polyclonals. As we passed the virus through Vero, by the time we got to the fifth or sixth pass, we started to see a change in the reactivity, and that change became static after about ten or 11 passes and didn't change after that.

Three of the monoclonals which I highlighted there went flat negative, nothing, and one of the monoclonals changed the staining pattern. The polyclonal antibodies we used, the rabbit polyclonal antibody, we had a chicken polyclonal antibody made in chickens, they also reacted with the Vero, there is no question about that. But the monoclonal antibody pattern changed.

Interestingly, the neutralizing monoclonal antibody immunofluorescence pattern changed, it disappeared.

DR. HANON: Can I add a single point, because I think it is a very important point. It is about the validity of the assays that have been used.

In the two experiments I showed in terms of infectivity, the second experiment, which was a titration

assay, it was about the use of that particular non-antibody against PCV1 containing Rotarix. We detected positive staining, which confirmed the fact that the polyclonal antibodies is indeed able to detect the PCV1 that is present in Rotarix.

DR. ALLAN: Can I ask you, did you titrate that antibody to see if the titer was different against the homologous to the heterologous?

DR. HANON: No, that has not been done.

DR. ALLAN: So it would have been one in 500 as opposed to one in 5,000. You don't know.

DR. HANON: No, but I would like also to add the fact that in the experiment presented, we tested the equivalent of one dose in 500 final container, 300 final container. We also performed one test with the equivalent of ten final container with the FT-PCR. It is not about antibody. Then we get a negative signal, which most likely is due to dilution, but it is consistent with the titration assay that we generated with the immunofluorescence assay.

PARTICIPANT: I want to add something more. Concerning PCV2, for instance, which is a related virus, we made a lot of novel clones against PCV2. Although polyclonals are cross reactive, you can see a difference in reactivity pattern in between the monoclonals.

So I think we have maybe a singular thing that your

virus is changing a little bit, but your polyclonals are still doing the job.

DR. DEBOLD: Thank you for that presentation. You said that seroconversion in pigs occurs at about 12 to 14 weeks of age?

DR. ALLAN: Yes, seroconversion in pig herds that we monitored in Northern Ireland in 19 -- whatever it was, 80-something. That was the general trend we saw.

DR. DEBOLD: Did that have implications for when we should be checking for the presence of antibodies in human infants?

DR. ALLAN: I don't know the answer to that. I would think not, no. Well, PCV2 infection is by general standards is quite a slow infection. It takes a time to take off. For disease production it takes a long time to take off. Seroconversion is normally two to three days later than say parvo or other virus infections. But regarding humans I couldn't comment. I don't know.

DR. STAPLETON: I think Dr. Friedland would like to respond to that.

DR. DUBIN: Dr. Dubin.

DR. STAPLETON: I'm sorry.

DR. DUBIN: In the clinical evaluations that I described, the serologic evaluation was done after the last dose of vaccine. So that would have been several months

after the first dose. So there was a fairly large time period between the first dose and the serologic testing. There were intervening doses as well. So I think that time period that Dr. Allan just mentioned would have been covered.

DR. ROMERO: A series of questions for you. Would you expect to find PCV1 in pancreatic extracts from pigs?

DR. ALLAN: Yes. Actually, I would expect to find more PCV2 than PCV1, but yes is the answer.

DR. ROMERO: So then the next question is, because pancreatic enzymes are used to supplement individuals that have cystic fibrosis, if these individuals were taking this for long periods of time, would this be an interesting substrate of individuals in which to test for antibodies against that particular virus, both PCV1 and PCV2?

DR. ALLAN: These children are being --

DR. ROMERO: Supplemented on a daily basis, that is correct.

DR. ALLAN: Yes, is the answer. They would be a good substrate to look to see if oral ingestion of PCV1 --

DR. ROMERO: So I propose a study.

DR. TSAI: A related suggestion. Because genotype 3 hepatitis E virus is also transmitted from pigs to people, although there is very little acute hepatitis E in the United States, seroprevalence studies show that there is actually a fairly high seroprevalence in some states, particularly hog

producing states. These were the individuals who would have had documented exposure to a swine virus and I think potentially would be a good set of subjects to look at.

Then from the perspective of immunocompromised patients, there are increasing reports of chronic hepatitis E infection in transplant patients. Again, I think that might be a group where you potentially would have histologic specimens as well to examine.

DR. ALLAN: I want to mention one more thing before I go, can I? PCV2 is in a lot of ways a very strange virus. But one thing which might be important is that to get good disease in pigs with PCV2, you have to infect them with PCV2 and then immuno stimulate them. We immuno stimulate them normally by giving them a secondary infection or by vaccinating them.

I don't know what the vaccination schedule for children is, but if you are giving them PCV1 and then giving them another vaccine, you are immuno stimulating them. I'm not saying it is dangerous. I'm just pointing out that for PCV2 in pigs, you will not get good disease unless you immuno stimulate them, not immunosuppress them, immuno stimulate them.

DR. STAPLETON: If there are no other questions for Dr. Allan, thank you for your presentation. The next speaker is Dr. Phil Krause from FDA CBER.

Agenda Item: CBER Assessment of PCV

DR. KRAUSE: What I am going to do from the FDA perspective is give you a little bit of an historical perspective on other times when we have needed to think about events that may have some bearing on the discussion today. Then I am going to also prescribe CBER's laboratory assessment of porcine circovirus in vaccines.

One of the first times we had to face an episode like this was in the early 1960s, when Sweet and Hilleman discovered SV40. At that time, it was found that formalin inactivation of inactivated polio vaccines didn't completely inactivate SV40. But SV40-free vaccine was produced as quickly as possible. Licensure of oral polio vaccine was delayed until an SV40-free preparation could be made. There was no recall of inactivated polio virus vaccines, but there was also no release of additional IPV lots until the SV40 was removed.

To summarize a lot of information, as a consequence of this, some of the IPV recipients seroconverted to SV40. OPV recipients, including those in the clinical trials who were exposed to the virus, did not seroconvert to SV40. There have been concerns about SV40 in human cancers, but overall the evidence doesn't support a role. To summarize a lot of data, there were substantial epidemiologic studies to look into this after this event.

I would like to also mention the situation with endogenous avian retroviruses in egg produced vaccines. This came up in the mid-90s when a more sensitive PCR test called a product enhanced RT test, free enzyme reverse transcriptase, was developed. The reverse transcriptase enzyme was present in all retroviruses, so the presence of RT enzyme suggests that retroviruses could be present. In 1996 then, this test was used to show that previously undetectable quantities of reverse transcriptase were present in some avian cell produced vaccines.

Additional studies showed this endogenous avian retrovirus was a defective particle and does not induce productive infections in cell culture. And of course there was also the long safety record of hen's egg produced vaccines, together with absence of potential harm to humans, which were important considerations. In the early 2000's, we had to consider the impact of transmissible spongiform encephalopathy issues on vaccines. The concern was raised that bovine derived materials sourced from countries with BSE or at risk for BSE could post a theoretical risk to vaccinees.

So as a solution to the sourcing of bovine derived materials used for vaccine, production was changed to countries which were not categorized as having BSE or at risk for BSE.

For products with potential exposure to BSE or bovine derived materials in countries with or at risk of BSE, risk assessments were performed based on maximum possible exposures, dilution factors, potential infectious doses, et cetera. Many viruses were re-derived to remove all doubt. But we were able to move forward from that as well.

Then I would like to mention the many discussions we have had over the last decade especially on cell substrates. We have had numerous discussions and advisory committee presentations on the introductions of new cell substrates for vaccine production. The discussion addressed issues posed by the tumorigenic phenotype, theoretical infectivity and oncogenicity risk of cell DNA or other cell components. This was a difficult situation, because we were dealing with theoretical risks in the context of introducing these new cell substrates.

These issues as we have introduced new cell substrates also were addressed by risk assessment. As you know, we have been able to move forward and start new kinds of cell substrates to produce vaccines.

There was a previous FDA evaluation that addressed porcine circovirus, and I just wanted to mention that, that was related to the pancreatic enzyme preparations that were just mentioned a few moments ago. There was an advisory committee discussion in 2008 of one of our therapeutics

advisory committees. This discussion was broad ranging. It covered all potential porcine viruses, although porcine parvovirus and porcine circovirus were recognized as the viruses most likely to survive the production processes of these products. The final solution of a lot of discussion was the potential for exposure to porcine agents was mentioned in the package insert of this product.

I am going to switch gears now and tell you a little bit about what we have done at CBER to evaluate the issue in the vaccines that we are discussing today. Our major scientific approach has been designed to try to understand whether PCV and rotavirus vaccines is likely to represent infectious virus.

We have used two approaches to try to get at this question. One of these approaches is a molecular approach, these molecular studies, and the other is cell culture studies.

The kinds of molecular studies that we have done have been addressed, confirming the finding of the presence of PCV nucleic acid in vaccines. We have wanted to independently determine how much PCV nucleic acid is in the vaccine. We have done studies to determine whether PCV nucleic acids in the vaccines are likely to be particle associated.

We also asked the question whether PCV nucleic

acids are present as full-length genomes, either in a vaccine or if we look at preparations that are enhanced for particles, whether we could find full-length genomes in those particles. Then we asked the question of how does the sequence of PCV1 from vaccines compare with the sequence of known infectious PCV1.

What we reasoned then is that if we had answers to all of these questions, if we could find full-length particle associated PCV1 sequences in the vaccine, it was very likely that those represent infectious virus. Then we went on and also did cell culture studies to ask the question more directly of whether vaccine inoculated previously uninfected cells show evidence of virus infection.

In our initial studies we used four conventional PCR assays. Some of these we developed ourselves and some we obtained from the literature. These are numbered here in correspondence with the next slide. As you may have seen in the previous diagrams, PCV has two major open reading frames. On the right-hand side of the circular genome is the replicase gene and on the left-hand side is the capsid gene. We used PCRs that targeted both the capsid and the replicase of the genome, and we also used PCRs of different lengths. Then when we did these studies we did sequencing and Southern hybridization to confirm our results.

This summarizes our conventional PCR results in

this slide here. The way that this experiment was set up, we tested individual vaccine lots in duplicate. We did three separate independent experiments which are labeled as extraction one, two and three, and used various of the PCR primers to study these individual extractions. We studied Rotarix and we also studied RotaTeq, and we also studied Rotarix spiked either into RotaTeq or into serum.

In this diagram, a red means that it was positive for PCV1 DNA. In other words, we found the appropriate band and were able to confirm that band by sequencing, and the negative means that we did not find the appropriate sized bands, and the ones that are white weren't done.

The findings in this experiment included that Rotarix was consistently positive for PCV1 DNA. RotaTeq was negative for PCV1 DNA to the limits of this assay's sensitivity.

That is one important thing to understand about these PCR assays. Very often the sensitivity of a PCR assay depends not on the number of copies the assay can detect, but how much material you actually put into the assay.

So in these experiments, we started with one cc of vaccine, but we extracted 100 microliters of that and then of the 100 microliters that came back from that, five microliters went into the PCR reaction. These particular assays started off testing only 1/200th of a dose of vaccine,

which means that even in a very sensitive PCR assay, the limit of detection was not as robust as what we were able to achieve later on in the slides that I am going to show you later, by testing a larger proportion of a vaccine dose.

All of our assay controls, which include negatives, extraction controls and positives, yielded the expected results. These findings of course are consistent with findings that have been reported by GlaxoSmithKline as well as by Dr. Delwart.

I told you that we wanted to see if we could find full genomes in the product. So we developed a PCR to get almost the entire length, 1638 nucleotide PCR, which gets almost all of the genome which for PCV1 is 1759 nucleotides. We detected and cloned this product directly from final Rotarix product.

Then based on the sequence of this, as well as sequences that Dr. Delwart kindly made available to us from his metagenomics studies, we had the entire genome of the virus from Rotarix final container available. Although there was some variability in the sequence, there were no nucleotide changes which had not already been reported in Genbank for wild-type viruses. This includes no changes in open reading frames.

So we reasoned from this that we were finding the full-length genome of the virus in the vaccine, and that the

sequence suggested it was similar to other viruses which were known to be infectious.

To follow up on these studies, we then developed quantitative PCR assays. In these PCV assays we used three PCV1 primer pairs and two PCV primer pairs. You can see here that for PCV1 we were targeting the replicase gene as well as the capsid gene. Both of our PCV2 primer sets targeted the capsid gene, but we used primers of different expected product lengths and a couple of these PCRs you already saw in the conventional PCRs, and they were adapted by using a probe to the quantitative PCR here as well.

We studied DNA extracted from vaccine samples. We extracted our DNA in two different ways. We did a total DNA extraction, in which we got all of the DNA out of an individual vaccine dose, or in some cases a proportion of a vaccine dose or sample.

We also did what we call capsid preps in our laboratory. What that is, is a preparation which enhances for particle associated DNA. We treat the material with nucleases, which get rid of any free DNAs or RNAs, and we also ultracentrifuged through a sodium chloride gradient, because only particles should then be pelleted by ultracentrifugation. We then removed the supernatant completely and then re-suspended back up to the original volume.

In the capsid prep, if DNA survives that, it is very likely that that DNA is associated with a particle, which survives the nuclease digestion as well as the ultracentrifugation.

Then we quantified the PCV DNA in these experiments by Taqman quantitative PCR.

These are the results that we got with Rotarix here, shown to the left. To walk you through this experiment, on the X axis I am showing results using the five different PCR reactions that we have. The three ones in black are for PCV1 and the two ones in red were for PCV2. With each of these PCRs, we then looked at the direct extraction as well as the capsid preparation. The direct is shown by open boxes, and the capsid extraction is shown by a closed bar here.

We tested two different lots in this particular experiment of the GSK vaccine, with Rotarix. In this case we used a full dose of vaccine in our extraction, which gave us a better limit of quantitation than we had in the other experiments for this, because we had a larger amount of DNA that was going into our extractions.

What you can see here is that both of these Rotarix samples were positive for PCV1 in all three of these assays, and both of these Rotarix samples were negative for PCV2. I am not showing all of the assay controls on this, but in

these Taqman assays we do mutation positive controls, including tenfold dilution series. All of these assays we were able to quantify down to at least five copies, because the five copy standard is positive, and it fits on our linear curve.

Not all the virus DNA is full length, though, because the longer the primer set that we used, the less total number of PCV copies we detect. However, at each PCR fragment size, if you compare the open and the closed boxes, most of the PCV1 DNA was particle associated, because the closed bars represent particle associated nucleic acid.

So using the longest PCR primer, we can say then at least in these vaccine doses, each dose contained around 100,000 copies of near full-length particle associated PCV1 DNA, with 10_5 being around 100,000 copies.

We also did some additional studies on Rotarix by using the more sensitive PCR. In these experiments, we found PCV1 and PCV2 DNA fragments in the Rotarix final container. We also found particle associated PCV2 DNA fragments in the Rotarix bulks. Our particle associated tests for PCV1 were negative on the Rotarix bulk, but further studies are ongoing there.

We attempted some studies on the GSK IPV containing vaccines. What I can tell you right now is that they are not conclusive. We tested the final container for PCV1 and PCV2,

and they were negative, but when we spiked some Rotarix into the IPV, we were not able to detect all of the PCV1 DNA that we knew to be in the Rotarix, that we found in the Rotarix, when we didn't spike it into the IPV. So this experiment is considered inconclusive, because there was some inhibition either through the extraction or the PCR.

So we are planning follow-up experiments. These follow-up experiments will probably be most readily performed once we have bulk samples available. There has been some delay in us getting the bulk, thanks to the eruption of the volcano in Iceland, as well as various import restrictions. But I am happy that we will be receiving the bulk; actually it is being delivered this afternoon, so we will be able to do these studies soon.

We also did infectivity studies for PCV1 on the GSK vaccine. There are a number of considerations in setting up infectivity studies. Speaking as someone who didn't work with this virus until about six weeks ago, this is not an easy virus to work with. The virus has to get into cells, it has to find its way from the cytoplasm to the nucleus. The cycle time can take quite a while some of the time. Unless the cell is in S phase, unless it is actively dividing and synthesizing its own DNA, the virus will not replicate. That is because it is a very small and simple virus. It only has a couple of genes, so it really is relying on the cell to

provide most of what it needs to make copies of itself.

As we thought about this, we recognized that since we are starting with vaccine samples, there was a risk that inactivation of rotavirus might also inactivate the PCV. So we wanted to make sure that we did something that would preserve PCV that was in the sample.

There was a theoretical concern, we thought, that bovine serum might contain neutralizing antibodies of PCV, although Dr. Allan tells us that it doesn't, and thus reduce the sensitivity of the studies.

We wanted to make sure as we were doing these infectivity studies that no exogenous PCV was being added to the culture. We recognized that based on the literature, different cell types have different susceptibility to the virus. We recognized also that if cells became confluent, the virus wouldn't grow even in susceptible cell types, so we had to make sure to try to keep the cells in growth phase.

Then of course another complexity of working with this virus is that the virus doesn't cause cytopathic effect, so you can't look directly at the cells to see if the virus is growing.

Our approaches to each of these problems were as follows. We used heat inactivation under conditions where PCV is resistant to try to get rid of the rotavirus. We actually did one other thing as well. We did not use GSK's

manufacturer supplied diluent for the rotavirus vaccine, which we recognized was designed to help keep the rotavirus more stable. We instead used medium in which we thought the rotavirus might be less stable when we were doing our heat inactivation.

We used immunoglobulin depleted bovine serum for our cultures, just in case the presence of antibodies in the bovine serum might interfere with our results. We used recombinant trypsin and otherwise pretested reagents to make sure that no exogenous PCV was being added to the culture.

We used swine tested cells for our experiments. It is not because we think that swine tested cells are better than other cells where the virus is known to grow. These were cells that we were able to get into our laboratory quickly and we were able to get to grow to large enough quantities that we could do a fair number of experiments.

We also have in the meantime done some experiments in PK-15. The virus does grow certainly at least as well in the PK-15 as it does in the swine tested cells. But we did these experiments in the swine tested cells.

Then to make sure that the cells stayed in growth phase, we were very careful to inoculate sub-confluent cells. Then we maintained the cells in growth phase by doing a sub-passage into a larger flask at day three in order to keep them growing and to have as much S phase time as possible.

Then we evaluated our cultures by quantitative PCR to detect virus DNA, which is a different readout than others have used.

Here is the result of our infectivity study on a couple of lots of Rotarix. Just to tell you a little bit more about how this is done, at time zero we took a single dose of vaccine and put that into a flask of cells. We absorbed that for a total of four hours, and then we removed the inoculum by washing three times to clean off the inoculum. Then we took our time zero after that wash.

What we are seeing here at time zero should represent DNA that got associated with the cells during that absorption period. Everything else that was in the dose of vaccine presumably was washed away.

Then at day three we took a very small aliquot of this out and the rest of it ended up staying in the culture through this sub-passage. Then at day six we took samples from the then-larger flask to determine the total amount of PCV1 DNA that was present in the culture.

We evaluated mock infected cells as well as cells that were infected with two different lots of Rotarix, and we also evaluated these cultures by direct extraction, which is in the open symbols, and by the capsid preparation which is in the closed symbols.

You can see that the mock infected swine tested

cells yielded negative results. Cells that were inoculated with Rotarix showed increasing PCV1 DNA quantities, as measured by quantitative PCR. And because the closed symbols are also positive, the particle associated PCV1 DNA was also produced.

We also looked at the supernatants of these cultures. It is not shown on this graph here, but particle associated PCV1 DNA, including our near full-length PCR, was also detected in cell cultured supernatants at day three and at day six.

I also don't show it on this graph, but inoculation of cell lysates from day six. So we took the material from day six and we lysed that, and then we took the supernatants of that lysate and put that onto fresh ST cells to see whether we could transmit the infection to fresh cells, and that revealed a two to four log increase in PCV1 DNA quantity after three additional days in culture, which corresponds roughly with what we saw in our first three days of this culture.

It is important to note that we haven't formally evaluated the sensitivity of this assay, as in the case with many cell cultured assays, so we don't know exactly what it would mean to get a negative result in this assay.

The other thing is, I think one could imagine a lot of ways in which this kind of assay could get made even more

sensitive, including carrying this out for longer periods of time. As long as one can maintain the cells in growth phase for a long enough period of time, one should be able to get continued amplification of whatever is in the starting material.

To summarize our results and the results that have been reported to us by the manufacturers, we found PCV1 DNA in the product for Rotarix, and the manufacturer reports PCV1 DNA in the product bulks, seeds and cells, so these results are consistent.

We looked in a little bit more detail at the question of whether particle associated near full-length DNA can be found. Our answer is that it can be found. In cell culture, both we and the manufacturer report the presence of infectious PCV1.

For the IPV containing vaccines, all results are pending due to the difficulties we had with inhibition of PCR studies. As you will recall, the manufacturer stated that there is PCV1 in the harvest, the seeds and the cells, but not in the purified bulks or the final container.

Then for RotaTeq, the Merck product, we find particle associated PCV2 DNA in the harvest as well as PCV1 and PCV2 DNA in the final container. When we studied the non-particle associated DNA on the RotaTeq in the bulk, we had PCR inhibition, so we can't comment on that. The

manufacturer however does report presence of both PCV1 and PCV2 DNA in the harvest, and PCV in the final container.

What are the implications of all of this information? I wanted to present this slide to help lead into the discussion which is going to come up.

It seems clear that tests of increasing sensitivity can detect very low quantities of virus DNA in a virus. Moreover, we don't know if it is possible to completely eliminate PCV from porcine trypsin. Porcine and other animal derived reagents that are used in vaccine manufacture could potentially contain other viruses as well, known and as yet unknown. Of course, it is important to think about all this against the backdrop. It has never been possible to absolutely be sure that vaccines do not contain advantageous viruses.

So to conclude, our laboratory findings, PCV1 DNA, particles and infectious virus are present in Rotarix, PCV1 and PCV2 DNA is present at low levels in RotaTeq, with further evaluation ongoing. PCV1 DNA is present in polio virus harvests, although it is not detected by the manufacturer GSK in final bulks or final container. The quantities are low, and of course purification and inactivation procedures are in place.

I have a number of acknowledgements to make here. This work would not have been possible in such a short period

of time without an enormous amount of help from experts on PCV. That includes Gordon Allan and Steve Krakowka and Andrew Cheung especially, who I have had very useful discussions with, and who provided important reagents. X.J. Meng also provided us with some PCV1-free PK-15 cells, which we were able to use in some of our experiments that I didn't present here.

We have had an internal FDA scientific advice committee which has helped us work through the results here, but the real credit for everything that I am describing here belongs to people who work in my laboratory. In particular it is these two people at the top here, Christine Uhlenhauf and Shasta McClenahan, who have done the bulk of this work and have worked tirelessly, in many cases over weekends and practically around the clock in order to do a large number of Taqman assays. What I have shown you here is only a very small percentage of the large amount of data we needed to generate in order to be able to get these assays working, and to confirm the results that I have shown you here.

Thank you very much. That is my presentation. My next slide goes into the questions, but maybe you want to stop here if people would like to ask questions.

DR. STAPLETON: Yes, let's stop here, and then we will go through these after the open hearing. So are there questions for Dr. Krause?

DR. GREENBERG: Thanks, Phil, that was very helpful. Going back to the RotaTeq data, how far have you progressed in that vis-a-vis the size of the nucleic acid, whether it is particle associated? I assume from your presentation that that is just further behind, and that that data will come out in the next weeks or months or something like that in catch up with where you are with the Rotarix.

DR. KRAUSE: That data will come out. I can show you what we have so far, if you would like to have it on some backup slides. I think the problem with the problem with the RotaTeq is that the quantity of the DNA is a lot smaller, which makes it much harder to characterize what it is.

The other thing with the RotaTeq is that there is more cellular nucleic acid in the product, which leads to PCR inhibition when you test more concentrated samples.

DR. GREENBERG: Is it known whether the circoviruses are sensitive or not sensitive to low calcium environments? You can get rid of all the infectivity of your rotavirus by reversing, and if your circovirus is not sensitive, that is the fastest way to get rid of that part of your thing.

Then infectivity is the best way to detect low amounts of -- if it is infectious, it obviously amplifies. So the problem I assume is that the rotavirus there could mess you up, but rather than heat, if the circoviruses aren't

low calcium hurt, you just totally inactivate rotaviruses by EDTA. Just a bit of advice.

DR. KRAUSE: That is an interesting point. I don't know the answer, but I can discuss that with Dr. Allan afterwards as well.

What we did for this is, we took the vaccine and we re-suspended it in cell culture medium, which of course will have some calcium in it. The trouble is, I don't know the impact of trying to grow the cells in medium that has been completely calcium depleted would be.

DR. CHEUNG: Hi, Dr. Krause. Have you considered using a more sensitive cell line developed in Singapore by Professor Jimmy Kwan? He derived a cell line of PK-15 cells, and reported that regular PK-15 cells, the infectivity for PCV is appropriate ten to 15 percent pervasive PCV. But in the line that he developed, 100 percent is permissive to PCV. It would be advantageous to get that cell line to get a better sensitivity test.

DR. KRAUSE: I think that is right. What I don't know as we look at this is the relative sensitivity of different tests. One can do these kinds of sensitivity studies in cell culture and you can try to push the sensitivity farther. The more sensitive the test one can do, the better one's chances are of finding some infectious virus.

In this case, the ST cells that we used were sufficient to find the infectious virus in the Rotarix final container. What I don't know is whether using different cells would necessarily be advantageous over adding additional passages or things like that. But I would love to talk with you about that some more afterwards as well, if we can figure out how to get those kinds of cells in order to try to come up with more sensitive results. I think that would be useful.

What I don't know, and perhaps you can answer this, or Dr. Allan or Dr. Meng, whether that would be as sensitive as inoculating vaccine material into the pigs and see whether they seroconvert. If that is really the most sensitive way to look for this, we can come up with some answers in cell culture. But to really know for sure whether something is positive or negative, it may require an animal study, anyway.

DR. CHEUNG: Well, in terms of sensitivity, probably pigs as Dr. Allan said is more sensitive. But the thing is the time commitment in getting that and comparing to tissue cultured PK-15 derived cell line, you know it in a few days, very quickly. The other one will take a lot longer.

DR. KRAUSE: We can certainly look into that.

DR. HUGHES: If I understand one of the slides you showed about the increasing amount of viral DNA, that suggests to me if that is PCV1 DNA copies on your Y axis,

that the infectivity to DNA ratio in this is considerably lower than what we heard earlier. I think particularly if there are 100,000 DNA copies, is that -- am I making the right interpretation? Does this really suggest that less than 100 copies of DNA are infectious?

DR. KRAUSE: I have tried to make these calculations as well. On the Y axis, this is what we detected in the aliquot that we tested. This aliquot represented 1/80th of a vaccine dose.

So if you look at this and you say, we got close to 100 copies, just to make the math easy, DNA into the cells at time zero, you mutation that by 80, and that implies that in the entire flask, maybe we got about 8,000 copies, although it is a little less than 100. So it was 100,000 copies.

We may not have had a terrible infectivity ratio, at least in terms of what got into the cells. I can't promise you of course that all of these copies found their way into the cells. What I know is that they were associated with the cells and were resistant to washing away after the original absorption.

DR. HUGHES: So just to make this point absolutely clear, is this stuff that has been rescued once and then diluted, or is this stuff right out of the Rotarix sample?

DR. KRAUSE: It was straight out of the Rotarix vial, diluted in MEN, in media.

DR. HUGHES: So you have a much higher level of infectious virus in the sample than what GSK had, yes?

DR. KRAUSE: It seems likely, based on comparing the experiments. Although there are differences in how the experiments were performed. So I think it is very difficult to compare them as apples and oranges. But it does seem unlikely to me that if there were only three infectious copies per final container dose, that one would see this result.

DR. STAPLETON: You have not calculated the $TCID_{50}$ though, right?

DR. KRAUSE: No, we have not. We have started doing some dilution studies on this. We actually do have one experiment where we took a final container vial of Rotarix and diluted it one to 100, and asked the question of what we saw. In that, we have one experiment where we didn't see infectivity, and we have another experiment where we did. So I don't know if that is a question of needing to pit the experiments against one another and vote, or whether that means that perhaps the titer is close to around 100, and it gets diluted out.

We have not done detailed dilution experiments, and likewise we haven't either developed an assay which I am confident of it being sensitive enough to calculate a $TCID_{50}$.

DR. ROMERO: So following up on those questions,

these absorption studies were done at room temperature, correct? Initially.

DR. KRAUSE: This was done by the people in my lab. It was done either at room temperature or at 37 degrees. I think it was probably done at 37.

DR. ROMERO: So then as you have already inferred, some of this virus might have already gotten into the cell and uncapsidated. So the only way you are going to answer that question is to do these binding experiments at four degrees and keep the cells from internalizing the virus. Then you will know how much is actually present, at least virus is present on the surface of the cell.

DR. KRAUSE: I think we could probably do that. Because this represents all of the virus that is in the culture at our time zero, it would be a combination of what is on the surface and what is in the cell. So this would presumably be an upper bound on what could have gotten into the cell.

Agenda Item: Open Public Hearing

DR. STAPLETON: Thank you, Dr. Krause. I think we will move on. Next on the agenda is the open public hearing. I have some things I need to specifically say.

Both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering and decision making. To insure such

transparency at the open public hearing session of the Advisory Committee meeting, FDA believes it is important to understand the context of an individual's presentation. For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement to advise the committee of any financial relationship that you may have with the sponsor, its product, and if known its direct competitors. For example, this financial information may include the sponsor's payment of your travel, lodging or other expenses in connection with your attendance at the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships.

If you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

We have three people who have requested to speak. The first is Dr. Eric Delwart, representing Blood Systems Research Institute.

DR. DELWART: Thank you. In terms of conflict of interest, I need to report that I did have an hour or so long conversation with Merck, and they have promised me, I believe, a couple of hundred dollars. This was to better explain our experiments.

Here is the paper which will be in press. It will

come out, the final version, probably within a few weeks. I want to acknowledge Joe Victoria, who has now moved to Iowa. He has done the bulk of the work in about five months. Also I want to acknowledge Lawrence Livermore National Lab, who did the microarray work. They did that in record time. It took about I believe two to three weeks.

I just want to point out how impressed I am by the speed of the response to this article when it came out, first by GSK who rapidly confirmed the presence of the virus, the FDA and how quickly the FDA responded. It compares very well to the speed of review for NIH grants.

Some people have questioned whether I should have done this study; is it better not to know what is in your vaccine. I think it is a fair argument, but it is a big extreme. We should know what is in it. I think it is very important not to overreact to every little piece of DNA or animal virus that you might find in those vaccines.

A bit of background on where we have done this work. Even though I am affiliated with UCSF, my lab is in a blood bank, which is a small building in the heart of San Francisco. We share a building with a traditional blood bank; they draw blood and they test blood and they transfuse blood, and there is a small research institute.

My funding is basically NIH money as well as some soft money from Blood Systems, which is a very large

nonprofit blood bank, the second in the U.S. after the American Red Cross.

Now, because of the history of contamination of blood in the world, starting more specifically with the West Nile, then HepC, HIV and HepB, there is a high awareness of contamination and emerging viruses in the blood supply, which is why they fund my viral discovery program.

What allowed this methodology to come forth is basically the drop in price in DNA sequencing. Now using metagenomics, you can sequence pretty much all the nucleic acid in a given sample. It has led to a surge in new virus discovery, not only resequencing known viruses for studies in viral evolution, but also to explain many unexplained diseases.

This is the main focus of my lab. Why did we analyze vaccines? There was really no health reasons to analyze vaccine. It was more of a purely academic endeavor. The hope was to confirm that there really is no other viruses except the ones that you expect. So there were no health problems that needed checking.

There is also as we heard a history of bio contamination. There is SV40, as well as all the contamination of hepatitis B in yellow fever vaccines as well. There is of course huge public scrutiny of vaccine safety due to many cases of unexplained diseases.

So we decided to use metagenomics to look at the content of some vaccines. I went to the UC pharmacy and said, what live attenuated vaccines do you have this week, and I purchased everything that they had. We looked at live attenuated vaccines. We did not look at inactivated vaccines, reasoning that any efficacious viruses in an inactivated vaccine would be inactivated.

Here are some examples of the results that we got. We generate a lot of sequence data with methods which I will describe more following the next part of this meeting, about new technologies. But in Varivax, for example, I believe this is a Merck vaccine as well, is just the vaccine that you expect. That is all that you find. You find using similarity searches no other vaccine. You can cover the whole genome of the virus.

Here is another vaccine, MMR2. It contains three different attenuated viruses. Again you can find sequences from all three viruses, some more than others. Maybe that has got to do with the concentration of each of those viruses. Maybe it is the way we do the random amplification.

Here is the one that was a bit surprising, the presence of PCV1 in Rotarix. We did also find some rotavirus sequences in there. We found a lot more pig circovirus. That does not mean that there is more pig circovirus nucleic acid than rotaviruses. The way we do our random

amplification -- I will describe that a bit more in our next talk -- means that we may just be preferentially amplifying circovirus DNA viruses versus RNA viruses, suggesting rotavirus.

Of course, we have heard it many times this morning, this is not the first time, PCV1 is found in vaccines. In the literature you always find a lot of nice background. Using PCR, two animal vaccines were found to be contaminated with PCV1. This turned out to be pig vaccines, so I don't think people were too concerned if you find the PCV1 in pigs, but it is interesting nonetheless, and this comes up a lot. We heard that Merck's also has PCV1 and PCV2.

Again, we have heard that PCV, both 1 and 2, are extremely common in the U.S. I had a nice pork sausage this morning, and I'm sorry I ate some. It is also very common in stools. Seventy percent of pork products -- we only tested about a dozen pork products that we bought at the markets in San Francisco, but 70 percent of them were PCV positive. If you take stool from U.S. patients with diarrhea or from healthy individuals in Minnesota, you found about five percent of them also contain PCV1 nucleic acid. That by the way tells you that there is no great differences, that there is no association with diarrhea.

I tried to recruit some people for this experiment

here to measure the transit time of these viruses in the gut. After writing the IRB, I had no volunteers from the lab to do these experiments, so I decided to do it myself. I went pork free for a week, and then we tested it at a couple of time points, and it was PCV negative. Then for the whole weekend I ate pretty much nothing but pork, and what came out 24 hours later is PCV1 positivity in the stool. Then I stopped after two days, and a couple of days later the outcome was then negative. So it was the equivalent of a pulse chase experiment. My transit time was about 24 hours.

The conclusion to that is, PCV transits through the digestive tract. You can argue that it might replicate in the lymph nodes and so forth. We did a couple of experiments quite a few months ago to look at autopsy material from humans, not the gut but lymph nodes and other tissues, but these were formalin fixed so we could not use PCR very efficiently. But that would be one way to look for infectivity of PCV1, 2 or other related circoviruses.

If you look in stool, you find a whole lot of viruses. Should we be worried about all of those viruses? These were travelers with diarrhea in Nepal, and you can pretty much tell what they eat by finding the nucleic acids from different plants. If you look hard enough you will find viral nucleic acids in a lot of places, in a lot of different viruses.

Of course, this is probably the most important reason. You saw this slide this morning. This is the impact -- this just came out in *GID* a couple of days ago -- the impact of the rotavirus vaccines in the U.S., and presumably even more of an impact in other countries. Nearly 40 to 60 percent reductions in hospitalizations, and also evidence of a herd effect from vaccination. So this is an extremely important vaccine, I'll leave it at that.

Conclusions. PCV1 and 2 are often consumed in meat. It is not known, and everything I heard today confirms that, that it does not affect human. There are very effective vaccines. My recommendation is that to prevent shortage of these vaccines, that the Rotarix, the PCV1 and now the RotaTeq should be distributed. Their value is so much greater than any theoretical risk that it would be unwise to do otherwise.

That is the end. Thank you.

DR. STAPLETON: Thank you for your comments, Dr. Delwart. The next speaker is Dr. John Kolman, representing BioReliance.

DR. KOLMAN: Just by way of introduction, my name is John Kolman. I am head of R&D and genomics for BioReliance. I am hoping to give you a little bit of a commercial point of view on biosafety testing and how we are preparing to support the community, with or without slides,

in terms of this new PCV situation.

BioReliance is a company which is local, we are just down the street. We are local, but we have clients and also support facilities around the globe. We are large in number. We have been around a long time. We are mostly scientists. We build our own assays, we validate them, and we work to the specs that are required by our clients.

In terms of our facilities, we have both classic virology and cell culture suites, as well as standard nucleic acid testing facilities, and also a recent commitment to genomics. That is to say, those new cutting edge technologies that are going to make a difference for our clients going forward.

In terms of conflict of interest, I have not been compensated to appear here or to say anything. On the other hand, we work with the top 20 pharmaceuticals on the planet, and those include GSK and Merck, under a number of different contracts, a number of different circumstances. Everything that I am going to tell you here today is based upon internal work.

Just from a philosophical point of view, if you will permit me for a second, PCV is not going to be the first time that we are going to experience this kind of situation. The genomic tools that are becoming available to all of us now are such that we are going to be able to see things that

we haven't seen before, with sensitivities that we haven't seen before. I think it is important to be able to use these tools correctly, and also put them in the context of the biology, those points which we need to be concerned and those points which we don't need to be concerned.

So from there, I would like to point out four different lines of investigation that we are in the middle of or are prepared to offer at this point.

Let's begin first with standard conventional PCR assays, existing ones and new ones that we have initiated very recently. We are scrambling also to provide additional, what we believe are going to be germane assays based upon the shortcomings of Q-PCR, for example, and I will go through each one of those one by one.

On this slide, I am simply listing the assays that we have available right now. There are two, a qualitative and a quantitative. This is a gel end point assay available in the U.S. It is qualified. It detects PCV1 and 2, and its LOQ is at 100 copies.

This is a very important point for us. We are looking at this not as a test for PCV1, but rather for a range of related viruses that are likely to be important collectively, PCV1, PCV2 and likely some bovine as well.

There is a quantitative assay that is validated. This is available in the U.K. LOQ is also at 100 copies. It

does detect bovine as well. At the very bottom there is listed a automated quantitative assay which is being built by my group in the U.S. right now. When I say automated, I am simply referring to automated extraction and automated plate assembly for the Q-PCR. Its LOQ as it presently stands is 100 copies. We have taken our internal Vero cell line and we have done a 50 cycle Q-PCR using this test right here, and it remains negative, very clean, very nice.

The point that I would like to make here is that Q-PCRs are very good. You are going to see what you look for. That is to say, you are putting in very specific oligonucleotides, and if what you are looking for is there, you will see it. But if anything has diverged, you are not going to see it.

So to that extent, I would like to move on to the next point, which is detecting non-Genbank PCV nucleic acids, and how we need to be thinking about this going forward. What I am going to show you very quickly is just an investigatory test that we have done using massively parallel sequencing. This is our little moniker, MPS, for what is also frequently referred to as deep sequencing or next gen, et cetera. It is all the same.

What we basically have done is, we have done a Vero cell transcriptome, in particular hoping to find some PCV sequence. Importantly, MPS puts you in a position to detect

nucleic acids in virus and virions that oligo based assays might miss. I'm not going to belabor the point, I will also be presenting this afternoon, but basically are both cellular as well as viral transcripts, whether they are productive or latent, can be captured and then evaluated by MPS using standard methods.

That is really the easy part. Anybody can isolate nucleic acid. Anybody can push the button on a half million dollar machine. The real hard part is figuring out what to do with the huge amount of information that you get out the back end. For example, in a full transcriptome, if you are talking anywhere from 100 to 300 million base pairs, what do you do with it? The answer is, you have to apply very reasonable but rigorous bioinformatics. So we start with curated databases for adventitious agents, and then we have a process by which we remove those things that can be distracting, to the point where you get down to hits. The punchline is this. When we took our Vero culture with or without induction, listed here, and in the processing of this experiment we wash with HPSS and we use EEE to do a disassociation. This was not the norm for this culture in our hands. So basically we have been using standard porcine trypsin in the past.

When we did this analysis, out of 1.2 million reads, 317 million bases, there were exactly zero hits on

PCV. So this is consistent, though not below sensitivity of PCV certainly, but it is consistent that we are not seeing anything in our culture of Veros to be concerned about at this point.

On to the third point. This is also I think a reflection of Q-PCR assays. Q-PCR assays are going to tell you if anywhere from 80 to 300 base pairs of what you are looking for is present. It doesn't tell you if that is useful or helpful. What would be more helpful are these bottom two, where you can actually look for intact genomes or do interactivity assays directly.

To this point, in terms of molecular assay, we are in the process of developing our rolling circle assay. This is unintelligible to me from this distance, I can't imagine it being better for you guys.

But in essence, when you have a target which is circular, you can add random hexomers. You can apply a very precise polymerase to generate these extremely long single-stranded replicates of a circular template. You then go through the step where you generate the second strand. What ends up happening is that the concatimers line up thusly. They can be cleaved with a restriction enzyme, and you can score them on a gel based upon size.

What is nice about this, of course, PCV is a small ambi-sense single-stranded genome. This is perfect for this

kind of analysis. The nice part about it also is that it is extremely sensitive to a circular target that has to be closed.

This is some preliminary data. In here we are testing PK-15 that do have PCV1 in them, and also a PCV2 viral isolate that we acquired. I just want to point out here, rows 13 and 14, this is ten_4 copies of PCV2 and ten_2. This is dilutions down to ten_{-6} of PK-15.

What we are seeing here is, on this gel, you would have to say we are seeing 1,000 copies by this method, which is not bad, considering it is an RCA. But actually we have improved this since this point. Though 100 copies is probably going to be an LOD, the LOQ will be somewhere between 100 and 1,000, we feel. So this is something that we have put into our arsenal very quickly.

The last bit, Phil talked a great deal importantly about infectivity assays. Actually we all have been chatting quite a bit about infectivity assays. We also tried to put into place reagents that will allow us to provide that. It has been rather interesting for us. I would like to show that to you.

We had to start with acquiring the reagents needed. So first we acquired anti-PCV 1,2 antisera as well as PCV1 antisera so that you can distinguish between the two. They are polyclonal. Secondly, we obtained PCV-free PK-15 cells,

and we have confirmed both by IFA and by Q-PCR that they are in fact PCV-free. We have established both MCBs and WCBs for our own purposes going forward.

When the cells are taken and they are individually inoculated with PCV1 virus and PCV2 virus cultures, this is not Rotarix or anything, these are just cultures that we have acquired through traditional means, we applied the viruses, and after 24 hours we shocked with glycocyamine per the literature, washed, re-fed, maintained the cultures for at least 21 days, and *passaged* at least twice. So at this point, by virtue of the washes, the re-feeds and the passages, we are not expected to see any additional inherent virus, we believe.

The punchline is this, that when you infect with PCV2, you are able to see by IFA that it is positive using the anti-PCV2 and to sera negative with the PCV1. The same is true here for the PCV1. Using our qualitative PCR, we can distinguish between the two targets, using size discrimination.

Those data are all shown here. This is the PCV1 infection. These are the PCV2 antiserum, and you can see nothing except for the time-date stamp. Here you can see the cells showing up, nice whole cell stemming.

What surprised us a little bit about this is, this is after 21-plus days. So this is not a magnificent

infection, nor does it seem to be moving about for the culture, at least at the level of the quality of these reagents, of which we are not 100 percent sure.

We were pleased to be able to see the PCV1, but we were a little surprised. We did an additional experiment, where we took the supernatant from those same cells, concentrated on nucleic acid and then did the quantitative PCR. That is represented here. This is actually a 1/10 dilution of a very small quantity of input DNA into this PCR. There is a ton of nucleic acid floating around in the soup of that infection, which was kind of interesting to us. It is important to have a PCR end point, but it almost suggests that you have got a low level of infectivity, but those infected guys are high producers, which might be a way for us to put together a reasonable assay using this system.

That's it. Conclusions, simply that we have qualified as well as validated PCV assays which are available immediately. We have our eyes turned toward the future in terms of generating these sort of additional relevant assays for a full viral genome sequence, as well as infectious particle detection.

Again, getting back to the philosophical notion, this is going to likely happen again. Our ability to put these kinds of assays into place for additional things that we see based upon metagenomics and other things that come to light I think

is going to be very powerful.

Thank you very much.

DR. STAPLETON: Thank you, Dr. Kolman. Our next speaker is Dr. Barbara Loe Fisher, representing the National Vaccine Information Center.

MS. FISHER: My name is Barbara Loe Fisher. I am co-founder and president of the National Vaccine Information Center, a nonprofit organization founded in 1982. I have no financial conflicts of interest.

On March 22, FDA officials adhered to the precautionary principle and recommended that doctors suspend use of Rotarix vaccine after a private lab identified DNA from a pig virus in Rotarix vaccine, and the manufacturer confirmed the seed stock was contaminated, too.

As of yesterday, we know that DNA from two pig viruses, one of which has been linked to a wasting disease in baby pigs, has been identified in RotaTeq vaccine. In addition to a pig virus DNA that is not supposed to be in RotaTeq, the private lab reportedly also identified DNA fragment from a virus similar to monkey retrovirus in RotaTeq.

However, as yet no action has been taken by the FDA to again appropriately adhere to the precautionary principle by recommending that doctors suspend use of RotaTeq vaccine until the agency finds out where the particular contamination

came from, whether it poses a health hazard and why it was not detected by the manufacturer until now.

The FDA approved manufacturing process for RotaTeq and Rotarix allows use of African green monkey kidney cells, cow serum and a pig pancreatic enzyme, which presents opportunities for adventitious agent contamination, with for example the prion that causes mad cow disease or DNA from viruses that infect pigs and monkeys.

If the vaccine screening technology used by the FDA and vaccine manufacturers is not state of the art, the public cannot rely upon assurances that vaccines have met the government's legal requirement for proof of purity. The most troubling question that remains is how DNA from an animal virus could contaminate original seed stocks of Rotarix and evaded testing prior to the vaccine's licensure in 2008, which means that every dose of Rotarix given to more than one million children since 2008 was contaminated.

Were RotaTeq vaccine stocks contaminated with PCV1 and PCV2 as well? And why does RotaTeq vaccine have allowable thresholds of residual monkey viral DNA? And could there be other animal virus DNA in rotavirus vaccines that still has not been detected with currently used technology?

A February 2010 FDA document lists non-binding recommendations for vaccine makers, and states that vaccines should be free of adventitious agents, because residual DNA

might be a risk for causing cancer or being infectious. Parents being told by federal health officials to give their babies three doses of live rotavirus vaccine before six months of age expect those vaccines and others to be free from adventitious agent contamination, because the FDA legally requires vaccine manufacturers to adhere to binding regulations rather than offering companies non-binding recommendations.

The National Vaccine Information Center urges the FDA to one, recommend the suspension of the use of RotaTeq vaccine until more is known about whether PCV1 and PCV2 contamination is a hazard to human health over time, and Merck can guarantee that RotaTeq is adventitious agent free. Two, institute a legal requirement for vaccine manufacturers to immediately notify the FDA of any and all potential contamination issues. Three, raise the legal standards for testing of vaccines for adventitious agent contamination prior to licensure.

Thank you.

DR. STAPLETON: Thank you. Is there anyone in the audience who wishes to speak at this time? If not, I think for the sake of time, we will address discussion point questions one and two after lunch, and then save question three, which relates to this afternoon's topics, until following our discussion of questions one and two.

So we will be adjourned for one hour. We will start back at 1:25. Thank you.

(Whereupon, a luncheon recess was taken at 12:25 p.m., to reconvene at 1:29 p.m.)

A F T E R N O O N S E S S I O N (1:29 p.m.)

Agenda Item: Committee Discussion: PCV1 in Rotarix

DR. STAPLETON: I would like to call this afternoon's meeting to order. We have changed the agenda slightly because of prolonged discussion this morning. It was a very good and helpful discussion, I think. So what we would like to do now is discuss the first two discussion points related to the product-specific part of today's meeting.

So I will read the discussion points, and then what I might do is go around the table and ask people for their comments on each of these discussion points. There is no

voting today, there are no voting questions.

The first question is, based on the information presented today on the detection of PCV or PCV DNA in U.S. licensed rotavirus vaccines, we should discuss the available scientific evidence, identify facts to be considered in assessing the potential risk of the use of U.S. licensed rotavirus vaccines, and discuss additional scientific studies and information that we would recommend the FDA consider in future deliberations.

I think I will start today with Dr. Gellin, if you would, please.

DR. GELLIN: Sure, I will. Thank you, and thank you for putting together this conversation.

I think that we have heard a lot about PCV. It was a virus that I had never heard of until six weeks ago. Based on what we have learned from the literature and what we heard again today, there doesn't seem to be evidence that there is human infection. I think to the degree to which that can be reinforced, that I think is really going to be important.

Secondly, and maybe we can call on some of the other virology experts here, there is also the issue of whether or not this might cause something down the road. I don't know enough about virology to get into this, but would encourage discussion about what we know about viruses that have latent effects, transforming effects, to have some

conversation about why this virus does or doesn't fit into those categories.

DR. ROMERO: Let me begin by congratulating Dr. Krause on the body of information he put together in such a short time. I think it is nothing less than heroic, and it really shows what can be done by this sort of a group. So thank you for doing that.

I think we have data to suggest that this virus is probably not pathogenic for humans. Whether it can affect humans still remains a question, but it looks like it doesn't cause disease, if it does.

We certainly have a lot of data to support the validity of the vaccine, in other words, what it has done to the disease that is a major problem around the world and in the United States. So we have got a lot of good data that the vaccine is useful, and we need to keep that in the forefront of the discussions.

There are certainly more studies that need to be done. We have populations which we can begin to look at. I will throw out that issue of cystic fibrotic children that are receiving pancreatic enzymes from a very early age that have been receiving it for a longitudinal period of time.

Something to look at would be, first of all, is there PCV in those preparations, is the PCV infectious, and screening of these individuals for serologic markers of

infection, to see if they have antibodies to these. So I think we have got a ready population that we can begin to look at.

Then continuing studies on the virus that we currently have undertaken to understand how much of the virus is actually there, can we get any more information on the block to replication, that is, do we have any information to say that this virus isn't encapsidated, that there is a block to replication at some point that we can feel more comfortable about being a correlate for humans.

I think I'll stop there.

DR. TSAI: I am impressed by the ubiquity of this virus in foodstuffs. I didn't quite catch the relative exposure that we all have to the virus through foodstuffs versus the amount of virus that might actually be in the vaccine.

Granted, infants normally at this stage to receive Rotarix wouldn't normally be consuming pork products, but still, for the general population it would seem to me that it is quite possible that our overall environment exposure far outweighs the amount of virus that would be present in two or three doses of the vaccine. So I would be interested, and perhaps it is even known, just to have that fact.

I would support Dr. Romero's suggestion to confirm further whether the virus can actually infect people through

serological studies.

I'm sorry to mention this again, but because hepatitis E viral infections, infected people presumably are directly infected from exposure to swine or swine products. That would be a group where we know there is documented exposure, probably in a very similar way. Those national studies were conducted by CDC, and those serum samples may be available

Of course, transplantations who are documented to have chronic hepatitis E infection in Europe and in the United States, some of them may have tissues that could be examined by immunohistochemistry.

Those are just some suggestions for further studies.

DR. DESTEFANO: I think from both preclinical and postlicensure data that we have on this virus, both those vaccines seem very safe. The question is, is there any condition that we need to consider for more long term effects.

Similarly, for any kind of long term effect, is infectivity a requirement, where you would see any kind of long term effects. There may be other data that might be useful in that realm, might come from occupational studies, occupations that have been exposed to pigs, has there been any long term follow-ups of their health, might be something

to consider.

DR. GILBERT: My overall interpretation of the data is that they are robust and plentiful to demonstrate a favorable risk-benefit profile with a good safety record and a strong support for efficacy and effectiveness. I haven't seen any data suggesting harm from this PCV1. So I think the benefits weigh in favor of using these vaccines.

Because I am a statistician and I do some decision analysis as part of my work, I will mention that -- and this may be something that FDA does as a matter of course, but I think a decision analysis by the FDA or the collaborators could be useful for helping demonstrate the value of an approach that would not pull the Rotarix vaccines, as opposed to pausing them. And basically decision analysis would have to find some variable of interest that is of public health importance, such as cases of disease and deaths, subtracting out adverse events or serious adverse events due to the vaccine.

One would have to use the real data to date to try to inform the assumptions that would inform that decision analysis. But then one could demonstrate the strong utility of an approach that would keep the vaccines in the field. That is a rational way to support that approach.

I also wanted to mention something related to Dr. Dubin's talk. He talked about GSK's examination of the

presence of PCV1 DNA in stool samples in 80 volunteers. There was a preliminary examination comparing the solicited adverse event rates between the PCV DNA positive infants who got the vaccines in randomized trials versus the infants assigned placebo.

I think there is some value in expanding that analysis and trying to make it well powered to see if there is an elevation of these solicited adverse events in the PCV DNA positive kids compared to placebo. That analysis would have to be done carefully, because it would need to adjust for covariates that would predict the serious adverse events, so a cautionary analysis. You might need some sensitivity analysis to see if bias could be influencing the interpretation.

DR. STAPLETON: You raised something I wanted to raise. It seems a little bit surprising to me that such a small proportion of the vaccine recipients actually had DNA found in their stool. It would be interesting to look at why some do seem to transiently have virus and others not, but I will mention it more later.

DR. HUGHES: I would echo what my colleagues have already said, and say that I think the evidence is quite strong that there is no untoward event or disease in the short term. There is also no question that the vaccine works well. It is an important way of reducing morbidity and

mortality.

I would be a little more cautious in interpreting the data in terms of exposure by eating pork, because in most parts of the world pork is carefully cooked before it is eaten. Although this is a tough virus and it is reasonably heat resistant, it is inactivated by temperature, so I am not quite convinced that the fact that people eat pork and don't show bad problems is a strong argument for giving live virus to them.

The unknown then is what happens going forward, and whether this virus poses, instead of any kind of risk in the short term, any kind of risk in the long term. The fact that it poses no risk in the short term is certainly comforting, but I don't think that necessarily says that it is risk free in the long term.

I think the only obvious thing to do at the moment is to monitor those carefully who are exposed. I would hope that both the companies and the FDA would make an effort to keep track of people that are exposed, and if possible in particular the people
that showed transient virus.

I was very comforted to hear that GSK was planning to go forward with making a clean vaccine in as timely a way as they reasonably can. Obviously as they say, that won't be a fast process, but I think that is a perfectly reasonable

thing for them to do.

DR. STAPLETON: Dr. Baylor, would you like to comment?

DR. BAYLOR: Not at this time.

DR. STAPLETON: And Dr. Krause?

DR. KRAUSE: Not right now. We will reserve our comment.

DR. STAPLETON: Dr. Cheung, would you like to comment?

DR. CHEUNG: From the data in the literature and those presented here today, indicates to me that PCV1 does not appear to cause any disease in human. But in the literature there is some data that suggest that certain human cells can be infected by PCV. However, in that situation the data shows that it is an aborted or non-productive infection.

Another thing we need to consider here is we really don't know the long term effect of PCV infection in human cells, especially in the context that PCV produces a large amount of DNA. Its interactions with the host cell are effectively unknown.

In pigs we know that PCV does persist at a low level, so it is important to in the future monitor what is actually happening in a long term situation in humans. Mostly pigs go to market in a very short period of time, and we don't want it for years and years on end, but for human we

are giving it to infants, we have a long term to deal with. So I think that should be taken into consideration, the long term effect of this large amount of DNA present in human cells, even though they may be non-productive to produce infectious virus.

DR. STAPLETON: Are there any studies that you would like to recommend that haven't been suggested so far?

DR. CHEUNG: I just said that we have to monitor the long term interactions. Maybe that is integrations, maybe something of that sort, to see how long it persists in human cells.

DR. COFFIN: On the whole, the vaccine has what seems to be a spectacular efficacy and safety record. I think the loss of that vaccine in a very short time would be fairly devastating to a substantial part of the world. So certainly on the whole I would support continuing it. In the meantime, as expeditiously as possible cleaning it up, going back to scratch and developing clean seed stocks and so on.

There are a few things that should be followed up on more. Dr. Delwart presented a case study, an auto case study, where the peak of activity was one day. In the studies he showed based on the retrospective analysis of the clinical trial, the earliest time frame was three days.

It may well be that many of those would have been positive at one day if they had looked. What you are seeing

is that in a few of them they are tailing off after a few days. I think some more detailed kinetic studies to see exactly what is happening there.

I would feel very differently about these if for example there was a very large peak at one day and then it is just tailing off and tailing off, and there is never any sign of anything coming back up again. I would imagine that it wouldn't be that hard to design studies in which daily stool samples were taken for a week or something like that after a vaccine dose.

The other kind of study that it seems to me is needed is a better understanding of what is happening long term in some model or another. I think in humans it would be almost impossible to tease out long term effects of the vaccine if we had no hypothesis whatsoever as to what those effects might be. The only way to generate such a hypothesis would be from more thorough animal studies than have been done so far.

I would strongly recommend doing more careful pathogenesis studies on PCV1 than at least I have seen. I don't know everything that has been done, I have only seen what has been presented here, but with long term follow-up and perhaps also in a few other special kinds of cases, for example, in pregnant sows to see if there are any teratogenic effects, and a few other obvious things that one would look

at in terms of possible bad outcomes, to see if there is anything that could be used to more specifically assess in vaccinated populations.

So those would be my recommendations.

DR. MC INNES: In following up on the comments about the beautiful work that the CBER colleagues have put on the table, I personally just wanted to acknowledge my appreciation for the very clear and transparent actions of GSK. I think it was really very refreshing to see this open display of the problem, tackling the problem, very good approaches to trying to generate data as quickly as possible, and I am very grateful for that honesty and transparency with which this problem has been approached.

Life is a series of exposures. I think at the end of the day, does this new information substantively change the risk-benefit ratio. I think that is confounded by perhaps looking ta the U.S. situation versus other countries. I don't think this is easy.

I am constantly reminded when these things happen that we are dealing with the world of biologicals. This is not chemically synthesized, this is not a drug. It is very difficult. With bringing new technologies to bear vis-a-vis 3,4,5 sequencing, we are going to be finding things, there is just no question about it. Does that mean you ignore it? Of course, not. It just moves the goal post. I think we have

to keep striving to get it as close as possible at any point in time with the knowledge that you have.

I think the new information has to be addressed. I think it becomes very difficult in trying to integrate that into informing the public or consumers about what the reality of this is. Look at how we are struggling with this. I think it is very hard to deal with as a parent, for example.

I applaud again the transparency and the expression of moving towards a PCV-free vaccine. I don't think we have a choice, we have to move in that direction. The question is timing and how we manage it in between.

The question about U.S. licensed rotavirus vaccines. I feel a little bit uncomfortable. We know more about GSK's vaccines and we don't know very much about what is happening with the Merck vaccine and so on. I am a little bit uncomfortable about the way that is phrased and how to think about the contribution of the finding of PCV2 versus the finding of PCV1 alone. So I am wrestling with that a little bit, and whether this apparent quantification issue has relevance or not.

DR. STAPLETON: Are there any studies that you would like to recommend?

DR. MC INNES: I endorse what I have heard. I don't have any additional studies.

DR. WHARTON: I would agree with everything said by

the other committee members going around the table. I would like to echo the comments complimenting the really great work done by both our colleagues at FDA as well as the scientists at GlaxoSmithKline, in terms of very rapidly moving to get as much information as possible about this new and unexpected finding. I am astonished that there was as much to present today as there was, given the very short time since this finding first was reported.

The work today is helpful in how to make that risk assessment. The only thing actually that I was looking for that really didn't come up in the discussion today had to do with some of the issues about inactivation of porcine circoviruses, and specifically about susceptibility to irradiation. If in fact we think that porcine trypsin may be a source, it seems like that is a key question, and perhaps that is known by someone, but I don't remember hearing any discussion about it today.

As far as specific outcomes that may be worth thinking about, I am still a little concerned about Kawasaki disease. In the clinical trials done with both the U.S. licensed vaccines, there was an imbalance in Kawasaki disease cases between the vaccinated and unvaccinated arms. That is a disease that we don't understand its causation. It may be worth going forward to think about that and to see if there is additional work that could be done that could help

elucidate that.

That's it.

DR. SANCHEZ: I want to agree again with what everyone here at the table has said. I really have been very impressed by the efficacy and safety of the vaccine so far in the quite large clinical trials. So that to me brings the question of risk-benefit in a benefit standpoint for continued vaccination in the use of at least the GSK vaccine.

I agree again with the others that -- and I know that work is going on, to remove the PCV DNA from these vaccines. Certainly I think that is a goal that has to be achieved. Then if we continue to use these vaccines, I think that further studies should be done on these recipients, looking for shedding as well as any immunologic antibody production.

I think we have a cohort of babies who are easily accessible and can be further looked into, because it seems like this is just the start. I think that is where this problem is going to be -- I don't think this is going to be unique, so I think we should use this as an opportunity also to acknowledge the fact that it is probably safe, anyway.

DR. GREENBERG: An advantage of coming late in the turn here is that most every good idea has been said.

By way of transparency, I just want to make it clear that I played a personal role in developing earlier

versions of rotavirus vaccine and am somewhat in love with them. So my point of view needs to be taken with a grain of salt, although I have no personal financial relationship to either of these products.

I think the rotavirus vaccines, the data that Umesh showed, which is even more convincing to me, the benefits are so big, the risks would have to be immense to begin to outweigh those benefits. Virtually we see no real risk, and we have a very minuscule amount of theoretical risk at that point. So I think any look at this point would say the benefits far outweigh the risks.

That said, I don't think that the story has been told, or I understand the story as well as I should. I am certainly not scientifically convinced that infection with -- first of all, if the PCV virus is in this vaccine and we can really say to ourselves it doesn't infect people, the chances of it having a negative effect are reduced substantially more than if it does. Even if it does infect people, I'm not sure what the risk would be. But if it doesn't infect, you could still make up a story, but it would be a crazy story.

But we haven't really seen that. We have seen serology that has not been optimized. It is one serologic test. As all of you know, with modern techniques you can make better and better serologic assays. There is lots more sera in the bank.

So the first recommendation I would have is to optimize serologic tests as best you can, and then go back to a large number of people who have gotten this vaccine and say, can I see any immunologic evidence of infection.

Number two, the stool shedding. Maybe I misheard it, but I saw the experiments that Eric did, where it looked like his transit time was way fast. We saw kids that are shedding on day seven. Usually children have a faster transit time than adults.

So I am not totally convinced that we don't have even in this little bit of study some evidence of, maybe there was replication going on. So I would do more studies of people who get the vaccine. Just as we heard, children are getting vaccinated all the time. You can do serial stool specimens, and if you see a signal that increases, that is a sign of replication, and we should do that.

There are other ways of getting at whether this is just input virus or not. We heard a very good experiment where you could do the ratio of Vero cell nucleic acid to PCV nucleic acid, and that that ratio changes after you ingest it.

Finally, I would say the place that we could look most carefully to see whether there is any unintended risk, severely immunosuppressed children are not supposed to get the rotavirus vaccine, but we have already seen that they do

from time to time, because they are not diagnosed in time, because of errors. Those represent places where if this virus is going to infect anybody, it is going to infect the severely immunosuppressed child. So those would be places where those opportunities happen, unfortunately, that we should look extra hard and say is there any evidence of infection in those cases.

I think that type of data could easily come in in the next -- considering the large amount of vaccination that is going on in the United States and in Europe, in the next six months you will have a lot of answers there. That would increase my comfort level even more, if I knew that infection wasn't happening.

Then finally, I would say that of course, in the end cell substrates for vaccines, we need to move towards modern technology, so that in the future, GSK, Merck and everybody else has ways of better insuring absence of adventitious agents and their cell substrates.

I think some of you may not realize how much work will be involved in GSK's making a new vaccine. This is a Herculean amount of work, and will not happen in any rapid time frame.

DR. LARUSSA: There is very little left to say, but I will put my vote in that I didn't hear anything that makes me worry about short term safety concerns.

Having said that, I did want to make a few points. I think the information that was presented about the reactivity of the polyclonal antibodies with the PK-15 virus is comforting. I would still at least like to see some data that human sera reacts with the Vero cell adapted virus.

I think the prospective studies and long term studies on shedding in stool need to be done. Some of us have already started to try to figure out where our stool samples from our immunocompromised patients who shed rotavirus are, and we are going to find those.

I guess having said that, a couple of things come to mind. One is, I am left with this uncomfortable feeling about the story with PCV2 and the need for immune activation. I think I need to understand that better. It brings me back to the situation we have with for example some of the human parvoviruses, where the effects are either due to the co-infection with helper viruses. Then the other thing is, what is the promoter of this virus doing. Again, the example of the parvovirus, where the promoter for parvovirus can at least in vitro turn on genes for TNF alpha and for IL-6. So those last things could be looked at in vitro.

All in all, I am impressed with the amount of data that was presented today, but I still think it needs some tweaking.

DR. DEBOLD: I would like to echo the comments of

other people at this table, recognizing the impressive effort that has been expended to run additional analyses and pull together this meeting. I especially want to compliment the FDA for making the decision that I am sure was difficult, to suspend use of the vaccine while you were doing an investigation. Making a decision like that based on the precautionary principle in the face of uncertainty I think is the right way to go about public policy.

Which brings me to the few little comments that I have. While at the population level, looking at risks and benefits is an easier story than it is when you start looking at individuals who could potentially be affected. I am impressed by the number of gaps in the science and in the literature. There are a lot of gaps in evidence. I think making assumptions around those gaps, and assumptions that lead one to the conclusion that this is safe, and it is safe for all infants, I think is potentially erroneous. I think we will be putting some infants at risk.

Particular gaps in evidence that I think need to be filled have to do with the degree to which PCV1 and particularly PCV2, which we haven't had a lot of information about, is infectious. The extent to which they are replicating, I'm not at all comforted by the fact that several days out, you don't find evidence of the virus in stool or serum, in the absence of taking a look at where it

may be in other tissues, have we actually looked at lymphoid tissue. Those biological studies I think need to be done.

The other comment that I would like to make is that there are variations in human responsiveness. There are variations on the basis of genetics, there is variation on the basis of fundamental health status. The clinical trials data that we saw today, those samples are just too small. They really are not looking at the children who may be at most risk.

I think we need to look beyond just the kids with the severe immune diseases, but there are a lot of other children who have borderline immune status problems. They are premature, they have evidence of a number of neuroimmune conditions that may put them at risk, but they are not necessarily at severe risk. We have to look very, very carefully at the kids that are at the edges.

So I would say my general take on the evidence that is available to us is that it is colored by a great deal of uncertainty. I'm sorry for the policy makers that are going to have to go forward and make decisions, but I think that the right thing to do in the face of uncertainty is to make decisions that err on the side of caution.

Lastly, I am very concerned about the information that was provided about co-administration of PCV1 and PCV2 followed by some type of an immune stimulating event such as

vaccination, and that needing to be more or less the conditions that result in wasting disease in some pigs. This is something that thoroughly needs to be investigated before we give another dose of vaccine to a child that may actually have that same kind of an outcome.

Thank you.

DR. STAPLETON: Thank you. The advantage of being last is, I can agree with all of you. These are difficult. Both the FDA and GSK have done a tremendous job in getting a lot of information to us.

My take echoes many others on the committee. We have a very well documented benefit of this vaccine in children, and to remove that benefit based on the safety data would seem to outweigh the risk of continuing until an expeditious improvement cell substrate could be made. But that is a policy issue that will be difficult for the FDA.

I think that may be our next discussion point, so if we could move to the second discussion point. Yes, Dr. Gellin.

DR. GELLIN: I don't know if you want to invoke another round of this.

DR. STAPLETON: Sure.

DR. GELLIN: But since I went first, I did want to reflect on some of my comments.

Pamela raised one of the issues with the question

about vaccines, plural. The question only asks about risk. Fortunately everybody besides me was able to look at that more broadly and discuss both the risks and the benefits.

I think that Harry outlined that the evidence of the risk is zero to theoretical, and Vicky has given us a number of places where we can think where some of that theory may apply.

But the other part of the question is, this is an FDA committee. We are advising the FDA about U.S. licensed vaccines. Umesh did a nice job of portraying the global situation, but we also have to recognize that every country is going to have to make these as well. We are sensitive that discussions that happen here have impact in other places, and we are very aware of what this disease means in developing countries where they don't have access to medical care and where dehydration is often a death sentence.

I think we need to keep that in mind, and remind ourselves that as this discussion has gone here, where there has been a great discussion and we have gotten into a lot of the depth and some of the theory of things that need to go forward, that we also need to recognize that other countries are going to have to make these benefit-risk analyses on their own.

DR. STAPLETON: Absolutely. Other discussion before we move to the second question?

If not, let's go to the second discussion point. Given the available data about porcine circovirus, including lack of known infectivity and pathogenic effects on humans, and that circoviruses or PCV DNA may be present in U.S. licensed rotaviruses, we should discuss factors that need to be considered in determining whether or in what circumstances benefits of using rotavirus vaccines outweigh the theoretical risk of PCV.

Many of us have touched on this, but let's try and be specific in our responses. In fairness, I'll start with Vicky, or Dr. Debold, this time.

I think this gets to the policy issue, what circumstances do you as a committee member feel influences the risk-benefit of allowing these rotavirus vaccines, plural, to be started back in the U.S. for Rotarix or continued for RotaTeq versus not, until there is a different cell substrate.

Dr. Baylor, would you agree?

DR. BAYLOR: Yes, that is correct.

DR. STAPLETON: Dr. Debold.

DR. DEBOLD: I think that given that the vaccines are contaminated, I think this gets into the realm of, people need to be fully informed. This is truly an informed consent issue. I think we are still dealing with a lot of uncertainty here.

I understand at the global level that it is important to -- the reduction in hospitalizations, the reductions in cost, those are impressive outcomes. But the risk is going to be assumed by the individual, not society as a whole. So I think that kind of thinking and analysis needs to go into whatever policy it is that you come up with.

DR. LARUSSA: I think we sort of answered this before, but I will restate it another way. I think after this morning's discussion, the benefits of using both vaccines outweigh any theoretical risk that I have heard about. I think additional studies need to be done to reinforce that, but at least at this point in time, I don't see anything that would make me want to change my mind about using the vaccines.

I think the other thing I would add is, we have a pretty good amount of clinical trials and postmarketing data that points to continued safety.

DR. STAPLETON: I think Dr. Debold's point about informed consent and labeling -- actually, Dr. Greenberg is ready to pass on that one, so go ahead, Dr. Greenberg.

DR. GREENBERG: I couldn't agree more. Of course, in all medical interventions, information is a critical issue. When you are dealing with recipients who can't make their own informed consent, it is even more important. So information is incredibly important.

As I said before, I come out saying that for me, one, we have to tell people what the possibilities are. But if asked what I would do as a physician, I think the benefits outweigh the risks on an individual basis, as best I can predict.

I would say again that I would feel even stronger about that if I had better data proving to myself that PCV is not infectious for humans. That doesn't absolutely eliminate risks, and I don't think the data we have had to date proves that.

DR. SANCHEZ: I again want to agree with what has been said. In essence, I want to reiterate what I said before, that I do think that the risk-benefits far outweigh the benefits at this point.

I agree that the finding of the PCV in these vaccines will have to be stated and explained to the parents, which is going to be a major hurdle, but it has to be done. I do again feel that prospective studies involving serology and shedding on recipients should be done.

I also wonder if some studies looking at autopsies of infants who received the vaccine but have died from other causes, maybe that is another way of doing PCV of different tissues and seeing whether that could be also trying to see the invasiveness of this.

There were several infants who died with sudden

infant death syndrome. Those get autopsies. I wonder if there could be some national study looking at trying to get some of these tissues to study.

DR. WHARTON: This is an issue of comparing very clear benefits against risks that are as best we can tell theoretical, based on what we know. But of course we don't know everything. If the studies that have been suggested or are already underway and have been suggested today are done, I think we will know more.

But based on where we are with current knowledge, to me the known benefits clearly outweigh the risks.

DR. MC INNES: The vaccines that resulted in the very robust and impressive efficacy in observed safety databases are manufactured this way and contain these adventitious agents. So for better or worse, I think we have enormous confidence about efficacy and the observed safety for those parameters that were measured.

We have new technology that helped us find something. I think part of the problem is that we don't fully know some of the nuances and subtleties that we might be looking for if we had better understanding about the pathogenesis of an associated disease.

I bring back to the table that I feel more comfortable about the PCV1 than I do about PCV2, primarily because of association with etiology of the disease in

piglets. I think we haven't seen a lot of data about that. So I do feel discrepant concern about those two entities.

I think the benefits are clear, but as I said in response to the first question, I think the enormous challenge is being able to communicate what those benefits are from what hypothetical risks might be, and for a parent to be able to in an informed way weigh that and make a decision in a setting where access to medical care may be easy versus a setting where it may not be.

DR. COFFIN: Like everybody else, I agree with essentially everything that has said in front of me.

The issue of informed consent I think is very important. What is going to be extremely difficult, particularly in the present atmosphere of all this, which has eased perhaps a little bit but not a lot, antagonism to vaccines in general, probably for not good reasons, and the amount of dis-information that is going to be out there about this and freely available to all is going to be as large if not larger than the amount of information. So informed consent is going to be a major issue to be dealt with. I don't think there is any choice.

I also second the point that was just made about relative comfort with PCV1 and PCV2. We didn't hear much about PCV2 in the vaccines, obviously because it is a much more recent observation, and there hasn't been time to do the

kinds of workup we heard from GSK. But I think we really need to know a little more about that and the infectivity of that virus, what is really there, particularly given the possibly different pathogenic potential of those two viruses. So I have some nervousness about that one as well.

But on the whole, certainly at least for the GSK vaccine, I think the benefits strongly outweigh the theoretical risks that we know.

DR. CHEUNG: To me, this discussion is basically on the risks and benefits comparison. Although we have seen a lot of data, it also points out the gaps in our research and also the information we have, however, based on the information available today, and FDA has to make the decision based on much of this information, it seems to me that the benefits quite outweigh many of the theoretical risks.

So it seems to me that pulling these vaccines would create immediate problems with the immunization.

DR. STAPLETON: I think I will skip the FDA unless you have something specific, and we will go to Dr. Hughes. Of course, the FDA always has the last say.

DR. HUGHES: I am very much in agreement, particularly with what was just said. I feel really quite comfortable based on what we heard today about the immediate risk from the PCV1. That is not to say that I wouldn't feel reasonably comfortable with PCV2 if I saw more data, although

like my colleagues I am always going to be more skeptical of an entity that shows pathology in its normal host species.

I think part of the question is -- and I certainly also agree that in our current circumstances, in which we have an obvious benefit, where we have both morbidity and mortality that would be lost if we lost the use of the vaccine, we would have to have a reasonably compelling reason to stop. I think the real question then becomes, what sort of compelling reasons are out there that would make us stop, or at least make us suspend what we are doing.

I think that is the reason that keeping a very careful and close eye on the vaccinated population and keeping track of as many of those individuals in as careful a way as possible can be very useful. If there were evidence of infection, or if there were new data that showed there was some obvious untoward effects in that population, I would certainly want to very carefully reconsider what I just said. But based on the fact that the data very strongly suggest that there is not a substantive risk, -- that is not the same as no risk -- not a substantive risk in the short term, and that there is an enormous benefit, we have to go with the benefit and accept the risk.

This is one of those unfortunate situations in which you are not given a good choice and a bad choice, but you are given two difficult choices. But I am very much

convinced, given the success of the vaccine, that continuing based on what we know today, what I have heard today, it is the right answer.

DR. GILBERT: One way I think about this question is, if a decision analysis were done to help compare the utility of different policy decisions, what factors would we want to make that decision analysis robust.

I think these comments will end up agreeing with what others have said. One factor is, we need to have good data on vaccine effectiveness going forward. I think the data in the recently published papers about the rates of severe morbidity and mortality going down after the vaccine introduction are important, so it needs to be a priority to make sure those studies continue in a methodologically sound way.

Other inputs we need for a valid and rigorous decision analysis is, like I was just saying, we need rates of infection, if any, by these PCV viruses in the infants, and we need the data on replication if any, and we need a better powered assessment of the rate of serious adverse event in infants that have some DNA detected as PCV virus. We need a comparison with a control group like placebos to understand if there is some elevation.

It also might be worth looking at the PCV viral load levels and see if that has any effect on the risk of a

serious adverse event.

DR. DESTEFANO: I'm not sure I have that much to add. I still echo the less comfort with the PCV2, given that we haven't seen much information about it today.

Given the specific question, I might turn around and say there are specific circumstances which you might not recommend. The one that I have heard is severely immunocompromised infants. I think there are already precautions against that, so I don't know that it would require any change in current recommendations.

Then going back to thinking about outcomes again. When they first come to license vaccines and such, there are things that we do consider for long term, tumors and oncogenicity potential.

I was here when we first considered the vaccine last year. I'm sure that probably those kinds of studies in tumorigenicity, et cetera were done when we considered recommendations to license the vaccine. So I assume that it passed those tests or any immunogenicity testing that is done prelicensure. We have some data related to those potential outcomes.

DR. TSAI: From an industry perspective, companies are required to put together risk management plans. A component of that is communication to patients and practitioners. A number of people have mentioned how

potentially difficult that communication would be. I would agree.

I think industry certainly wants to work actively with the CDC, with the agency, to help communicate a consistent message, because it is a difficult message for the public to understand, whatever the actions may be.

If I could just backtrack a little bit on the first question, I would be curious what the survival of this virus is on environmental surfaces. It is a tough little virus. I was just thinking, how would I clean up a kitchen where I was preparing pork, and is it possible that it is very prevalent on environmental surfaces, so that even infants might be exposed.

DR. ROMERO: Let me begin by saying first thing, I was not minimizing the efforts made by GSK in my initial comments. Dr. McInnes is correct, your level of transparency is incredible. So that was an oversight, it was not a slight.

I think pretty much everything else has been said. I think there are some important things to stress, that is, the world view of this vaccine. We lost a previous vaccine that could have saved thousands of lives worldwide, and we need to keep that in mind in this particular vaccine. This vaccine has been shown to be very efficacious. In the developing world, those of us that practice there know that

diarrhea is a death sentence for many infants. We now have a way of preventing that death sentence.

I think as has been mentioned already, in what circumstances would you have more information in limiting the uses of the vaccine even further, if we can get more information from those individuals that are severely immunocompromised. I'm not talking about HIV positive individuals, I'm talking about those individuals that essentially have no immune systems. They are your SCIDS patients. It has been pointed out earlier that if we do a global search for these, we can find them, because the vaccine is available in a number of countries around the world.

If this virus follows the same trajectory that picoma viruses do, for example, polio virus, they will shed this for a very, very, very long time, if not years. So that would be a very good source to look for in individuals that have not been reconstituted immunologically.

The issue of informed consent is essential. I think that we the practitioners need to make sure that our parents understand what the new findings are and try to put them in a context of risk benefit as much as we can, given the limited time that many of us have with our patients.

I concur that the issue of PCV2 is still up in the air. We don't know a lot about that, and we need to try to

get more information about that. That is an area that we need to focus on.

I think my closing statement is going to go back to something that we learned 60 years ago. When the echoviruses were first identified, they were named as an acronym for enterocytopathic human orphan viruses. In their initial discovery, no pathologic condition could be isolated, could be identified with them. With more time, it became obvious that they were associated with conditions like aseptic meningitis that is generally benign.

I think we need to keep an open mind, and we are, I think the group has clearly opened that door, that short term outcomes are not the same as long term. We need to follow these patients over the long run.

DR. GELLIN: I will echo many comments about PCV2. Apparently Dr. Allan has got a two-hour lecture ready to go, and he has got his plane ticket in his other hand, so I think we do have to be mindful of that, so we will need to hear more about that.

A lot of the discussion was about communication and informed consent. We have had a very sophisticated conversation here. Ultimately this is going to boil down to some doctor talking to some patient about something they saw in a headline. So I think it going to be an opportunity to try to better understand that conversation and how it can be

truly informative, which is not going to be simple.

Like many things in this business, you can't script these. But today's MMWR which came out yesterday was about regulation vaccination coverage. So at least we have a baseline by which we can see how our efforts will go.

Thanks.

DR. STAPLETON: Thank you. Dr. Baylor or Krause, any additional comments?

DR. BAYLOR: No. We haven't heard from you.

DR. STAPLETON: I don't have much to add, or anything to add honestly, to the comments earlier. The only question I had is not specifically related to this. I think I addressed this in my initial comments on point one.

I think informed consent is critical. Personally I view the risk-benefit equation to be clear cut. As many have said, we are facing a theoretical risk with proven benefit for a serious disease.

The other question that I had that I thought someone would get to, and I heard someone briefly mention it, but one research question that I think remains is to better understand in pigs the DNA status, is it episomal, is it integrated in chronically infected piglets.

I heard a couple of side comments, but I think that is an interesting question. If it is an integrated virus, then that provides opportunities to study that in pigs.

Also, if it were infecting humans and integrating in pigs, it would presumably integrate in humans. We would be able to look at humans, in tissues, to look for integrated DNA.

Are there any other comments from the committee? If not, we will move ahead with our topic two, advanced analytical methods and the characterization of cell substrates. Dr. Keith Peden will provide us an overview of this topic.

Agenda Item: Advanced Analytical Methods in the Characterization of Cell Substrates

DR. STAPLETON: We will move ahead with our topic two, advanced analytical methods in the characterization of cell substrates. Dr. Keith Peden from FDA will provide us an overview of this topic.

Agenda Item: Characterization of Cell Substrates Used in the Production of Viral Vaccines for Human Use and Summary of New Technologies

DR. PEDEN: Thank you. I think I was going to say for inviting me, but this is a mandatory event.

I have two charges today, one of which is to try to tell you what we have been recommending for cell substrates and viral seed testing with respect to adventitious agents.

We thought it would be useful to go through the

history and tell you how testing is evolving, and in the second part talk about some of the new methods in summary form of what we are going to see down the line.

My second charge is to keep people awake, particularly those four people who came on red-eyes.

The summary of the outline. As I say, I am going to talk about approaches for the detection of adventitious agents, and talk about some of the recommendations that have been used over the years and appeared in the cell substrate guidance that has just been released. In the second part I will talk about some novel technologies for adventitious agent testing, and try to mention a few issues associated with each, and talk about some of the regulatory challenges. Then the committee, I hope, will give us some guidance about what are you going to do with the information.

I thought we should say first of all that vaccines are the most effective way to control infectious diseases. I think this gets lost in our era of vaccine coverage, and the infectious diseases have in many ways gone, and all we have to do is read Charles Dickens to find out how bad measles was in the 19th century. So I think it is important to realize that the vaccines are a very effective way to control infectious diseases.

In general, the safety record of vaccines is excellent. However, we need to address all these issues that

come up, because maintaining the public confidence in vaccines is critical to public health.

What is an adventitious agent? An adventitious agent is a microorganism that has been unintentionally introduced into the manufacturing process of a biological product. These include bacteria, fungi, mycoplasmas, rickettsia, protozoa, parasites, TSE agents and viruses.

We thought it would be useful to say what we mean by freedom of. For a substance to be considered free of an adventitious agent, assays must be demonstrated that a defined quantity of vaccine is negative for that agent at a defined level of sensitivity. That is critical.

The level of the assay sensitivity is determined experimentally using standardized reagents. Alternatively, and this is critical for manufacturing, a validated manufacturing process shown to remove an adventitious agent to a defined level may be used to demonstrate freedom from that agent. So the word free is in inverted commas because there is nothing absolute in life, as this meeting today demonstrates, in fact.

What we find is, adventitious agents are detected by a combination of methods and strategies and at various stages of production. This is a holistic approach that we hope that one technique may miss something and another one will pick it up.

This shows that we are not relying on a single approach or strategy. We hope that mutation strategies provide to the extent possible assurance that products are free from adventitious agents. As I said before, the manufacturing process is critical to result in high quality vaccines.

Here is the current FDA recommendations for cell substrates. It is rather long. You can copy it down and you can read it tonight.

Current methods for detecting adventitious agents. These are broad overlapping schemes to detect as wide an array of viruses as possible. So obviously you want to have a broad way of detecting these viruses. What is critical, and maybe most people understand here, but maybe not, methods evolved over time.

This in many ways recapitulates virus discovery. These were done in the past to discover viruses, and these were gradually, as the technologies become standardized and accepted, they became incorporated into the recommendations for detection of adventitious agents.

These infectivity assays are in animals and cell culture. We have lots of methods. We have the non-specific method, which will detect known and unknown agents, in animals and cell culture, and more recently in the molecular biochemical approaches, and then specific methods for known

agents and for families. These are molecular and mainly PCR now.

The non-specific methods. There are various animal models. We didn't want to go through them in detail, but manufacturers know they very well. Adult mice, suckling mice, embryonated hens' eggs, guinea pigs and rabbits. These tests were originally used because they detected viruses not readily detected in other systems. So they have evolved over time, and this is why they are in the recommendations.

Non-specific methods in vivo. I don't expect you to write all this down, but we thought it would be useful for the public to understand that these tests detect a wide range of viruses, both in mice and in eggs. There is a huge number of viruses up here. This is to try to tell people that these methods do capture a large number of virus families.

However, they have limitations. The sensitivity is unknown for wild type strains, as the methods are generally established with lab adapted strains. Many viruses pathogenic to humans do not replicate in rodents or in eggs, so they do have limitations.

There is another in vivo test, and that is the antibody
production test. The test article is inoculated into animals, an adventitious agent is detected by the presence of antibodies to that agent. These are mainly performed in cell

substrates when there is a possibility of exposure to rodent agents, but some do it even when there is not. These viruses are detected on this large array of viruses shown here.

More recently, the antibody production test seems to be being replaced by PCR tests to these specific viruses and more. So over time the antibody production test seems to be being replaced by the PCR test.

The non-specific tests in vitro. These methods are based on the ability of cell cultures to grow a wide array of pathogens. They are also based on their extensive use in diagnostic labs to detect human pathogens. There is a huge microbiology lab database for what cells can replicate.

The good thing about cell culture assays is, you can inoculate larger quantities of inocula than often in molecular tests. This increases the sensitivity of the assay.

The cell culture assay can detect a large number of agents, including cytopathic viruses, hemadsorbing viruses and hemagglutinating viruses. The selection of the cell line depends upon the potential of exposure to agents. The recommendations in the guidance is that species and tissue type of cell substrate, in addition human diploid cells and monkey kidney cells are recommended.

The readout for cell culture tests is generally cytopathic effects in culture of the viruses is cytopathic;

of course it is not, and there are other ways to detect it. Hemadsorption and hemagglutination is historically the way to do this. More recently other readouts are used, such as antibody staining and PCR, for these kind of cell culture assays.

Again, the inflammations of these tests are that they can only detect agents that can infect and propagate in indicator cells. The sensitivity is unknown for wild type strains, as the methods were usually established with lab enacted isolates. Many viruses pathogenic to humans do not replicate readily in cell cultures such as human papillomavirus and HCV virus, among others.

What are some other non-specific tests? People have used transmission electron microscopy. This can detect virus particles in cell substrate, including those from endogenous viruses. The morphology provides an indication of the type of viral contaminants. Different retroviruses can be identified and classified.

However, the problem with this assay is that it is extremely insensitive, and generally considered to require about ten_6 particles per ml to be detected. It is a quantitative assay, and a positive result would require additional tests, for example, PCR tests and infectivity assays.

Dr. Krause mentioned the reverse transcriptase

assay this morning. All retroviruses have reverse transcriptase in the virion, therefore these assays can theoretical detect all retroviruses. As Phil mentioned, the quantitative product enhanced reverse transcriptase study became available in 1996. We recommend these and most people use these, because you can quantify the amount of virus present.

The problem is, some cell substrates express non-infectious endogenous retroviral particles, and Phil mentioned some of those this morning. And because the assay is very, very sensitive, false positive signals are obtained from cell lysates. That means that if you get a positive under certain circumstances, you may need to do infectivity assays.

Then there are some specific tests for virus. Most people now look for specific viruses using the PCR test. It is usually limited to viruses that are of concern for a specific product. Additional testing is recommended by PCR test. Such tests are obviously based on the known virus sequence, and conventional PCR and quantitative PCR are used.

In addition, more recently partially degenerate primer PCR can detect members of a virus family; they are starting to be used. Examples of these, human pathogen and certain human tumor cell lines, for example, in lymphocyte lines, HIV, liver lines, maybe hepatitis C, et cetera.

Then when warranted, PCR tests of various animal viruses are recommended, again apropos today.

Now we switch to the second. I just want to mention some of these molecular methods of adventitious agent testing.

The strategy for adventitious agent testing is to divide it into sample selection, amplification schemes and detection methodologies. Among the sample selection, it can be either genomic DNA, cellular genome, transcriptome, the messenger RNA or the RNA transcribed, or viral particles.

The amplification scheme is family specific PCR primers and degenerate primers, et cetera. For the detection methodologies, one can use mass spectrometry, microarray technologies or high throughput sequencing as we have seen.

If you use cellular DNA, the advantage is that all sequences will be represented theoretically in that assay. Disadvantages, the complexity is very high, and therefore may be insensitive for virus detection.

The transcriptome has advantages that all sequences that are transcribed are represented. There should be a lower sequence complexity, and increased sensitivity of virus detection based on the reduced sequence complexity. However, the disadvantage might be that the results are cell cycle dependent for a different expression at different stages of the cell cycle, and also you get endogenous viral sequences

will be expressed. So dealing with that information may be complex.

For the viral particle, purification as we have seen from Dr. Delwart's work and Dr. Krause's work. The advantage is, the enrichment for viral sequence lowers the complexity, and should increase the sensitivity for virus detection. The disadvantage, this might exclude some viral sequences in fragile capsid. So if purification proceeds it disrupts the capsid, and then you treat with nucleases to remove the genome, and then you may lose some.

In the amplification schemes, PCR can be done using primers to amplify members of a virus family, so that advantage should amplify most known viruses. The disadvantage is that unknown viruses will be missed.

PCR using fully degenerate primers or anchored degenerate primers. All virus sequences should be amplified. The disadvantage is, the detection and identification are extremely complex.

The detection methodologies. There is at least one well-known method of mass spectrometry now. This can only detect viruses or virus families because the PCR primers were made to conserve regions of genomes. The sensitivity has not really been established.

Microarray methodologies. You can either use direct application of nucleic acids to the microwave. This

results in a lower sensitivity, but there will be no introduction of sequence bias by methods such as PCR, which can amplify preferred sequences. PCR amplification prior to application of nucleic acids can increase sensitivity, but the potential for introduction of sequence bias and PCR contamination could be an issue.

The most likely one that is going to get high visibility is this, the high throughput sequencing. You heard some technologies that BioReliance are bringing on as a contract lab.

There are several technologies now available for high throughput sequencing. We didn't think it was useful to go through all of those. New bioinformatic tools are required to analyze the vast quantities of data generated. This is usually not available to poorly funded groups like our own, but the expertise, the curated databases, standardized analysis methods and programs are required. This is really a sub-bullet of this. It is extremely complex to analyze this, as you heard today from BioReliance.

It can be applied to genomes, transcriptomes and viral particles, and was applied in the case of Dr. Delwart, but also new viruses have been isolated by this technology. The Merck cell carcinoma virus was isolated by exactly this, purified viral particles, and degenerate PCR and parallel sequencing.

The interpretation however may be unclear. There are vast quantities of sequence, but this might be difficult to analyze transcriptomes, likely to get large amounts of endogenous retroviral sequence and unclear how to interpret this in the absence of additional work.

Virus particles are likely to produce fewer sequences, but also could get uninterpretable results. However, over time as new viruses are discovered and more data are entered into databases, we expect that these issues of interpretability may be reduced.

How do we consider some of these things in the new generation of molecular methods? Things like the sensitivity is usually not determined for these yet. They do not indicate whether a virus is infectious. All we are looking at is the genome of a virus. It doesn't say anything about the phenotype of that virus or whether it is infectious.

Each different method may require different types of standardization and standards to be used in a regulatory context. This I think will take an enormous amount of work to get these assays standardized, much less validated. The breadth of detection has not been studied. The reproducibility of robustness is generally not known. Many of the techniques are not commercially available, although as you see, they are starting to become available at a price. And many of the results will require follow-up.

Some final considerations. Adventitious agents is always a continuing challenge. Novel viruses are being discovered and will continue to be discovered. If anything is definite, this is it. There is no doubt that we will get new viruses over time.

Many of these could be present as adventitious agents or cell substrates or biological products, and the new cell substrates that are being brought on line for insects, plants, fungi, et cetera will bring an additional set of issues that I'm sure this committee will address in future years.

What have I said today? I told you that adventitious agent detection relies on the use of mutation overlapping strategies. This redundancy is hoping to capture all of the agents, but of course it never is 100 percent.

Detection methods for adventitious agents continue to evolve, and represent improvements in technology, including sensitivity and throughput. These new technologies can potentially be a powerful means to support safe product development

However, there are regulatory challenges, and the committee I hope will give us some guidance on this. Evaluation of risk for new viruses detected by using these new evolving technologies may be complex. There may be no infectivity assays for novel viruses. Pathogenicity of

viruses often takes years to establish.

Then with the assays themselves, which assay do we use in standardization of these assays and validation of these assays? Then an additional problem may be that the harmonization amongst various international regulatory authorities, how do different countries regulate these vaccines based on the information that these assays can provide. So that is a serious question.

I would like to acknowledge all my colleagues, because no one gives a talk in isolation from the FDA. I got good guidance from these people.

Then I will turn it back to discussion point number three, which is this one on here.

Thank you.

DR. STAPLETON: Thank you, Dr. Peden.

DR. PEDEN: Keith.

Agenda Item: Open Public Hearing

DR. STAPLETON: Keith. Next on the agenda is the open public hearing.

Both the Food and Drug Administration, FDA, and the public believe in a transparent process for information gathering and decision making. To insure such transparency at the open public hearing session of the Advisory Committee meeting, FDA believes that it is important to understand the context of an individual's presentation.

For this reason, FDA encourages you, the open public hearing speaker, at the beginning of your written or oral statement to advise the committee of any financial relationship that you may have with any company or any group that is likely to be impacted by the topic of this meeting. For example, the financial information may include the company's or group's payment of your travel, lodging or other expenses in connection with your attendance at the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the committee if you do not have any such financial relationships.

Should you choose not to address this issue of financial relationships at the beginning of your statement, it will not preclude you from speaking.

We have six people who requested to speak. I would like to ask this afternoon's speakers if they would try to hold their comments to five minutes or less. We would appreciate it, as we have gotten behind schedule.

The first person for topic two is Dr. Eric Delwart, representing Blood Systems Research Institute.

DR. DELWART: I want to acknowledge for support NIH and Blood Systems Research Institute, which is always concerned about blood safety.

We do run a viral discovery program. As was mentioned, there will be many viruses discovered. As a

matter of fact, our small group has already found quite a bit.

I just wanted to add a few comments about experiments that might be useful to resolve the issue of PCV1. It is surprising to me that Vero cells can be infected by PCV1. It might be interesting to inoculate African green monkeys, from which Vero cells are derived, to see what cells are targeted. It could inform studies such as looking at autopsies from children as to what tissues might contain PCV1, if any.

The issue of trypsin also came up. That may be often contaminated with PCV1 or 2. I would suggest, why not use recombinant trypsin?

The current methods we just heard for detecting adventitious viruses are mostly focused on inoculation of animals or some cell lines, as well as some specific PCRs. These can be very sensitive, but they are limited to only a subset of the potential viruses that could contaminate any biological products. Considering the huge number of viruses out there, the majority of these viruses are probably not detected by most of these assays.

What other methods might be very useful to test the safety of biological products? In my mind there are two such methods. One is the viral metagenomics or the metagenomics alone, which is what we used, a massive deep sequencing

approach. Also microbial microarrays were discussed, also known as viral chips. There are quite a few generations of those out there that contain literally hundreds of thousands of probes.

Both of these methods start with the random amplification of the nucleic acid. It is very important that this sort of approach be optimized, and that the sensitivity of microarray or deep sequencing be determined and compared to PCR.

But fundamentally the random amplification of nucleic acids is required for both techniques, and is based on a primer which is pretty much a random sequence, but a set sequence followed by a stretch of eight and/or mixed bases. This through prime and the primer will sit theoretically anywhere on viral genomes or any piece of nucleic acid, and will extend off of that primer.

This is usually done with the R-T step, then a DNA polymerase step, so that DNA products with this sequence at both extremities are generated. Then you come in with just that sequence and do 30 to 40 rounds of PCR.

Then you take that randomly amplified product, you can either hybridize it through microarray or you can sequence it. You can sequence it with various technologies. We have been using pyrosequencing because it provides longer read lines, but there are a lot of progresses and other

technologies. Presumably any of those sequencing approaches will be valid.

For example, we had a very productive collaboration with Lawrence Livermore National Lab. Their microarray, which contains 380,000 probes, can target all known viruses as well as bacteria.

So metagenomics in a nutshell is the 4-5-4 sequencing machine to which you input this randomly amplified product. You come out with a lot of data. It is true that the bioinformatics is a little more complicated, but we did put a package together with a part time, very talented Dr. Chung Lin from Stanford. This bioinformatic package once assembled is publicly available, could be used by anybody, given that you have the computer power to push your data through.

This is an example where we multiplex about 40 different samples into one 4-5-4 run in order to save some money. The data that comes out can be assembled in context to get either the full viral genome sequences if you have a high titer viral stock, or a partial singlet sequences if you have a very low titer viral stock.

Then you can take all your data and you basically blast it, that is, you look for sequence similarity in various databases, viral databases, bacterial databases and so forth.

This is an example of a hit where you find a new

virus. That is, one of your sequence reads is aligned to the viral database. Positive hits are shown in red. You can just eyeball the sequence alignments, and you know right away that it is a virus that is 80 to 90 percent related to a pre-existing virus.

This technique is non-specific. That I think is the beauty of it. It may not be as sensitive as PCR at the moment. It depends on the depth of sequencing that you can go through. You can detect the viruses circled in red, all types of families, large and small genomes, RNA, DNA, circular, linear, fragmented, envelope, non-envelope. We discovered many known viruses as well as a few new ones. So this shows the non-specificity of the approach.

This is an example where you have a virus at a high titer. You generate the entire genome sequences that are aligned to see the depth of coverage. The fact that you have peaks indicate that your random PCR is not perfectly random. It is random enough to get the whole genome, but there certainly can be some tweaking and improvement on the randomness of the surrounding PCR.

This is an example of the rate at which new viruses are being discovered with this and other approaches. These are sometimes daunting, all these virus types. This is a breakdown of the virus family infecting humans. There are 23 families infecting humans of viruses. Some are populated

with only a single species, for example, hepatitis B is the only member of that type of virus which is a DNA virus with a reverse transcriptase stage. Other families such as the picoma *Viridae* family are populated with literally hundreds of different strains that should be grouped in different generas.

In red is our contribution in just a few years with just a few people of the diversity of viruses known to infect human. Probably we have increased by ten percent the diversity of viruses known to infect human.

It is a long step to go from finding a new virus to showing that it is a pathogenic virus. That requires much more work. That is why people, even if they do find new viruses, should not automatically assume that these are pathogens. It is most likely that we have identified all the nasty pathogens already.

The strength and weakness of viral chips. They detect all known viruses, and the key is known viruses, simple, sensitive, quick, inexpensive. You need to confirm your data by PCR. Otherwise you just have a hot dot on your microarray.

The microarrays do not detect highly divergent viruses, those that have not been discovered yet.

DR. STAPLETON: Dr. Delwart, could you sum it up, please?

DR. DELWART: To sum it up, there are two techniques that I think are quite promising for viral discovery and for checking the viral purity of products and vaccines. These are microarrays and bio metagenomics.

Thank you.

DR. STAPLETON: Thank you. Our next speaker is Dr. John Kolman, representing BioReliance.

DR. KOLMAN: Hello again. Let me thank the previous speakers, Dr. Peden in particular, for the excellent introduction to the complexities, the opportunities and the difficulties of implementing some of these new technologies and where the shortcomings can come into play.

When we began the process of establishing our genomics capability within viral lines, we went through exactly the process that was described by Dr. Peden. That is, we asked the question, to what extent can an array support our needs going forward and to what extent can we use something like the mass spec analysis approach to support our needs going forward.

The answer, we felt, was that since they were both oligonucleotide dependent, you are going to limit yourself to seeing what you have to see, rather than seeing everything. Hence the commitment to massive and parallel sequencing going forward.

The facility for massive and parallel sequencing

within viral lines is in the Rockville site. This is one of our several brand-new suites that we have built of late. It is a GMP designed facility, so we can support regulated flows as well as R&D endeavors.

The kinds of work that we are doing presently, we don't advertise this at all, in fact, the kind of work that comes to us rather organically right now typically falls into one of these bins, characterization of cell substrates in terms of new vaccine development, master cell bank and master virus stock characterizations. Something that we call internally fermenter fires, which tends to push everything else off of the queue. Typically a lot of the work that we get either is referred by other clients or by recommendations from some regulatory organization, that an analysis to a certain depth needs to be done, so people will come to us and say, what does that mean, and we apply MPS as appropriate.

In terms of the discovery that Eric has the fun to do, it typically comes to us in the form of fires. A fermenter will go down somewhere on the planet. All of the logical tests have been performed, nothing is coming up positive, what do you do next. What you do is, you start doing MPS. These are more planned endeavors where very specific questions can be asked about what sort of adventitious agents are present in a particular target.

One of the most important things that isn't often

mentioned that I do like to mention in these sorts of talks is that when you do MPS, it is different than doing traditional Sanger sequencing. Traditional Sanger sequencing is, you get a clone, you take that clone, you put it onto an NABI device, you get an answer. What you have an answer to is the sequence of a single piece of DNA from the original target. Whereas when you do MPS, there is no cloning step, and every divergent nucleotide, every subspecies, everything that is drifting in a population will be captured within the analysis, as long as it is about five percent of the population, a very important thing going forward, especially in terms of virus substrate characterizations.

There are a number of things that I would like to talk to you about. First of all, the fact that as Eric pointed out, these things are often done with a random primed amplification library. There are pluses and minuses to doing it that way. I would like to discuss that a little bit. And just point out at the very bottom that the GMP process, because we do do this routinely with our viral lines, is ongoing for MPS as well.

We chose to go with the Roche 4-5-4 platform for a couple of important reasons. First of all, you can flip the assay overnight. You get very long reads, which is nice in terms of associating SNPs or deviant genomes one from the next. That is the most important thing I would like to point

out on this slide.

What is important to us when we talk to a client and how we design experiments in a custom fashion are three important points. One, what is the source of the nucleic acid, two, what sort of amplification method will be used, if any, and finally, the bioinformatics.

In terms of the source of the nucleic acid, this splits you into two different spaces. One is a traditional transcriptome type analysis or by comparison, an amplicon analysis. These are two very different approaches.

MPS, whether you are running a solid or a 4-5-4 or whatever is meant for is to do genomic or transcriptome analyses, where you have a very large complexity library of nucleic acids and you are trying to get as broad a snapshot of all that material at one point.

That is how all the library chemistries are designed. We will do that as need be, especially in the case of characterizing the cell substrate.

By comparison, if you are analyzing culture fluid as Eric has done and as we do for clients, and also as part of our internal investigations, you are talking about a very low complexity pool of nucleic acids. That is not appropriate for these machines typically. That is a very small amount of nucleic acid. You do have to amplify it and do so in an agnostic a fashion as possible in order to make a

library that is going to make a difference for you. So this becomes very important.

The transcriptome analysis is very straightforward. You are collecting the cellular transcripts as well as any viral transcripts, whether they are productive or latent. You collect them, you extract them, you have transcriptome MPS. In the case of a cell free or an amplified analysis, you would take the supernatant from a culture, which would include any fragmented nucleic acids that might be released from dying cells as well as the virion. You would do a nuclease step to remove as much of the contaminating material as possible, and then just extract the nucleic acid from the virions and proceed from there. It is at this point that you choose to apply an amplification step.

Now, amplification steps. As Eric pointed out, the one that is typically used these days is a random amplification using this hybrid structure here. Also as Eric pointed out, this generates hot spots in the course of your analysis, depending upon what you are looking at and which genomes you happen to be targeting.

There are other approaches that are in the literature, including those that Phil Krause has developed, which help to reduce and modulate this activity. We feel that going forward what is going to be the best for all is to be able to capture targets in a family specific process going

forward and this is what we are working towards very carefully right now.

Why bioinformatics? This is the hard part. As I mentioned this morning, it is very easy to push the button on a machine and walk away and come back and be very satisfied, because you have all this data, but what the heck are you going to do with it. One overnight run on the 4-5-4 results in enough data to populate 30,000 pages of text overnight. So we will do this several times a week.

What we really do is to reduce this down to ten pager memory stick. To do that, we have the bioinformatics and statistics needed to do this. This is done in a very custom fashion, depending upon what the client is asking us to do, or what our internal initiative is requiring of us.

Typically in the case of establishing a new substrate for vaccine production, if you have an overnight transcriptome, one that is going to give you 100 million bases, what you need to do is, you need to very quickly reduce that down to something that is meaningful to you.

We do that through a couple of different steps, establishing certain cutoffs with the quality of the read, the length of the read. We have internally maintained curated databases for adventitious agents which we update on a weekly basis. Then we have a process to remove those things that can put you off the track. There are a lot of

things which are riffraff that you don't need to pay attention to, and that is done here.

In a typical example, and this is nobody's data in particular, this is an analysis where were evaluating not necessarily a vaccine substrate in this case, but a B cell line. 200 million base pairs were recovered, 5,000 reads were blasted against our viral database, and pretty much all of them were dismissed either as false hits to BVDV or ERVs.

The question of sensitivity with MPS is a hoodoo guru for all of us. It is very difficult to capture by virtue of the number of variables that are involved in performing the assay. It is still much more of an art than a science, quite frankly. However, when one does a transcriptome analysis, you can rely upon things that you might do if you were doing reverse transcription. That is to say, if I have a housekeeping gene at a very low level, do I detect that or not.

The answer is, we will see with good reliability a housekeeping gene that is present at about 100 copies per cell as defined by the microarray people. That is the point at which we work, at least in the case of transcriptomes.

What gets really fun, as we have seen, is when you start seeing new things. We have initiated an internal process to evaluate FBS serum from a number of different suppliers and a number of different lots. What we find is

that there are a fair number of bovine viruses that come up, either at low or high levels, depending upon which lot you are looking at, and also some new things which we are in the process of writing up as well.

MPS for our clients. The way we are trying to position it is intended to not only do the virus hunting as need be and the cell line characterization as need be, but other things that we think can fit into the process very comfortably, something like genetic stability. So building a cell line and assuring that you have got a certain number of copies as well as a certain number of integration sites can very comfortably and easily be performed in a single MPS run by targeting specific sequences.

DR. STAPLETON: Can you please sum it up for us? Thank you.

DR. KOLMAN: I am very close. Just to recapitulate something that I mentioned earlier, MPS by virtue of how the library is formed allows you to find low frequency variance very comfortably, especially if you are at about the five percent level.

That is just a summary. These are the people who have contributed to this body of work, in particular Colette Cotet and her collaborators. This morning, much of the work was done by somebody who unfortunately I don't have listed here, Dr. Audrey Chang, who has been very helpful in pushing

for the virology part of this for us.

Thank you very much.

DR. STAPLETON: Thank you. Dr. Michael Hantman, representing Charles River Laboratories, will speak next.

DR. HANTMAN: Hi. I work for a company, Charles River Laboratories. We do biosafety testing, biological pharmaceuticals. Our clients are vaccine manufacturers, but Charles River paid my expenses to come here.

My focus is from a GMP client perspective. We do testing, and it has to be GMP compliant. So I want to be clear that I am not opposed to technological advances in analytical detection methods such as the metagenomics and the large microarrays. I think they are great technologies.

Metagenomics is very powerful. I think it will potentially generate a lot of these scenarios that brought us here today. You still need to decide which adventitious agents pose a threat.

We have a current assay. It is a quantitative PCR assay. The reason I am pointing this assay out is because it is GMP compliant, it is a validated assay based on regulatory guidelines. It exists today. It is specific for porcine circovirus types 1 and 2, so it is slightly degenerate in that it picks up those types, but it doesn't pick up every virus in the world, that is true.

This assay has been validated. It is a rapid

assay. It can be completed in one day. It is specific, targeting the replicase coding regions in both PCVl and PCV2. It is a sensitive assay, one copy sensitivity in a background of 500 nanograms of cellular DNA. A reliable assay, no false positives, no false negatives necessarily, depending on the samples, but in the validation it was very nice.

Just to explain, due to recent events, we are having clients send in more samples for this PCV testing. This is just one example of the components of a viral vaccine. It was sent in recently, including a working cell bank that was Vero cells, some crude process harvest, and the master viral bank.

We performed this validated Q-PCR, targeting PCV1 and PCV2, and no viruses were detected, no virus DNA sequences were detected in any of these samples, which shows that mutation matrices can be detected.

Just to throw in this, this has nothing to do with the previous experiment. We just wanted to show that PCV can be detected in porcine trypsin. It is interesting that the levels are not all the same, depending on the supplier. You should test raw materials as well as final products to prevent these events from happening.

It is also important to talk about DNA extraction in any of these assays. You want efficient DNA extraction.

This shows a porcine circovirus, this happens to be PCV2, a dilution series of porcine circovirus. At each dilution step the DNA was extracted and then run through the validated PCR assay. It shows that both the PCR and the extraction are linear over a wide range.

Again, I just wanted to get out there that I am not opposed to new technologies, but in order to implement new technologies in a GMP environment, you have to validate the assay, you have to look at the sensitivity, false negatives, false positives, robustness, the ruggedness of the assay, and it is always good to have follow-up assays to confirm that positives are true positives.

Thank you.

DR. STAPLETON: Thank you very much, Dr. Hantman. Our next speaker is Mr. Walter Kyle.

MR. KYLE: Good afternoon, everybody. I am Walter Kyle. I have sued the United States government regulatory agencies on a couple of occasions and some manufacturers along the way, but I don't have any really active cases, and I don't feel I have any conflict of interest. I cam here on my own expenses. I just wanted to make a few comments.

I would start -- the discussion you were having earlier on the committee about the benefit-risk ratio, there was a comment. In 1980 in the *Federal Register*, which I happen to have in my notes, concerns have been expressed

about potential oncogenic effects due to the presence in the vaccine of unknown viral contaminants or the genetic fragments of oncogenic viruses somehow incorporated into the genome of the vaccine virus. Such a theoretical risk should be given little or no weight when estimating benefit-risk ratio.

I think that is probably the position a lot of you have taken. But my comments, the comments I am prepared to make, follow from reviewing a lot of documents I have obtained in litigation.

Historically the Food and Drug Administration has shown little concern about releasing live polio vaccine grown in substrate contaminated by African green monkey herpes viruses. The Division of Biological Standards found 100 percent African green monkey polio vaccine substrate contaminated with a simian herpes virus in 1972. Lederle's response, the cytomegalovirus contingency plan, outlined Lederle's plat to block regulations that might eliminate African green monkey cytomegalovirus from its vaccine.

No regulations were enacted. The FDA and chairman of the American Academy of Pediatrics Infectious Diseases Committee at the time, Sam Katz, apparently agreed with Lederle's position that cytomegalovirus could not jump spies from African green monkeys to humans.

The Coburn strain of African green monkey

cytomegalovirus biopsied from the brain of a North Carolina child with encephalopathy put the lie to that position in 1978. The discovery surprised no one, because in 1971 AGM CMV was found to replicate in human cells and be the least species specific cytomegalovirus. The FDA never warned doctors or mothers about encephalopathy or other herpes related diseases. The Bureau of Biologics estimated oral vaccine caused six to ten deaths per year and 500 hospitalizations. At the same time, the package insert suggested a one in a million chance of vaccine associated polio and ten cases a year. It was not a warning, it was a warranty. It deflected issues and attention away from the live virus vaccines, the latent virus issues.

Cytomegalovirus is but one of a series of herpes viruses found in monkeys used to make live polio vaccine. Kaposi's sarcoma, herpes virus and Epstein-Barr virus naturally infect the African green, rhesus monkeys and chimpanzees used to produce the oral polio vaccine.

Autoimmune disease is associated with such herpes viruses including mutation sclerosis, lupus, chronic fatigue syndrome, Burkitt's lymphoma and now autism. But Sabin's polio vaccine viruses can carry genes from other viruses. The Division of Biological Standards identified that trait in 1964 and labeled it interdigitation.

Random recombination events during infection of

contaminated substrate allowed transmission of such viruses from monkeys to humans in oral polio vaccine, with the vaccine virus acting as a carrier or in the vaccine itself, which was not sterile.

In 1976 the FDA discovered unknown retroviruses contaminating polio vaccine, but released the contaminated vaccine anyway in 1978. In 1980 the FDA had justified its actions by stating in essence that the regulations did not require removal of the type C RNA containing retroviruses from live polio vaccine, because the reverse transcriptase test that won Baltimore and Tibben the 1975 Nobel Prize were not required by the 1962 regulations governing polio vaccine.

By 1982, two years later, an epidemic presentation of monkey viruses arose when Epstein-Barr virus was found associated with oral hairy leukoplakia, hairy tongue and Kaposi's sarcoma herpes virus simultaneously caused Kaposi's sarcoma in thousands of homosexual men who had presented with human cytomegalovirus disease. The epidemic was AIDS, and it has been suggested that it was a combined epidemic of HIV and KSHV.

I suggest that mandatory regulations versus non-binding guidelines requiring the elimination of any vaccine contaminant or vaccine virus contaminants within the actual vaccine virus itself, if not proven safe for humans, even if the testing techniques were not available when the

regulations were written.

I believe that the guidance for industry that I have attached as Exhibit 2 is a sham. It was written to insulate the FDA from liability under the Tort Claims Act, where discretionary decisions cannot be the basis of a lawsuit.

Finally, I feel the guidance, the relaxed approach to controlling the issues directly violates the mandate of 42 U.S. Code 262, that requires the Secretary to insure the safety and purity of vaccines.

Thank you.

DR. STAPLETON: Thank you. Our next speaker is Barbara Loe Fisher, representing the National Vaccine Information Center, founded in 1982. I have no financial conflicts of interest.

As has been mentioned today, contamination of vaccines with animal viruses is not new. In the 1950s and early '60s, polio vaccines given to millions of children and adults used monkey kidney tissue cells contaminated with simian virus 40.

In 1973, a prospective study of more than 50,000 pregnancies concluded that inactivated polio vaccines given to pregnant women in that study between 1959 and 1965 were associated with excess malignancies and brain tumors in children born to those mothers. If there continues to be a

dispute in the 21st century about whether monkey viruses or monkey viral DNA in vaccines can in fact cause cancer in humans, there is no dispute that polio vaccines were contaminated with the monkey virus.

Vaccine manufacturers are allowed to use cell material that comes from bodies of mammals, including humans, monkeys, cows, pigs, dogs and rodents, as well as cells from birds and insects in either experimental or currently licensed vaccines. There is always the risk of adventitious agent contamination that can escape detection.

The discussion last November in this committee involving a vaccine manufacturer seeking a license to use caterpillar cells which have the potential to be infected with insect viruses that are hard to detect to make influenza vaccine is indicative of how important the contamination issue is becoming, as hundreds of vaccines using novel substrates are being developed. Drug companies are experimenting with dog kidney and human fetal retinal cells, even though these cell lines have been documented to cause tumors in animals, and there have been discussions in this committee during the past decade about using cancer cells to make vaccines, even though there has been a longstanding prohibition on their use due to the risk for adventitious agent contamination.

The recent detection of DNA fragments from a bird

virus in measles vaccine by the same private lab that identified pig viral DNA in rotavirus vaccine brings to mind detection in 1995 by Swiss scientists of reverse transcriptase from an avian rotavirus in influenza vaccines, as well as the live measles and mumps vaccines using chicken cells for production.

Reverse transcriptase activity has been associated with the presence of rotaviruses that can permanently alter the genes of the cells they infect. This is no small matter, when the CDC now recommends that every American get an annual flu shot from six months of age through life. Even during pregnancy, the genetic and biological integrity of the unborn child developing in the womb may be exquisitely vulnerable to the effects of adventitious agent contamination of vaccines.

The contamination of seed stocks of the virus vaccine with animal virus DNA that was not detected pre or postlicensure is an important wakeup call for industry and government.

The National Vaccine Information Center urges the FDA to one, explore with vaccine manufacturers technology that does not rely on utilization of mammal, bird, insect or other living cells that can be contaminated with adventitious agents, posing a risk to human health.

Two, institute stronger legal requirements for proof that vaccine cell substrates and other materials used

for production of seed stocks are free from adventitious agent contamination and remain free throughout the manufacturing process before lots are released for public use.

Three, while vaccines are being thoroughly retested for adventitious agent contamination, the FDA should institute stricter labeling standards to fully and clearly inform the public using vaccines about residual adventitious agent content in all vaccines.

Thank you.

DR. STAPLETON: Thank you. Our next speaker is Dr. Elena Jouravleva, representing Beckman Coulter Genomics.

DR. JOURAVLEVA: Hi. My name is Elena Jouravleva. I am representing Beckman Coulter Genomics, and like my colleagues from Charles River and BioReliance we are providing services for our pharmaceutical clients, but my trip was paid by Beckman Coulter Genomics.

The main topic of my very brief five-minute presentation is to advocate the committee to consider issuing a regulation for the use of the next generation technologies for the initial screening of the vaccines. This particular technology allows us to answer the open question. We will be able to look for unknown instead of targeting presence of the known. Existing technologies are also highly susceptible to variation particularly in viruses and even in quantitative

PCR technology is highly sensitive. We are also offering it in the regulated environment. It is also susceptible to mutations which can happen in a viral population.

The same next generation technology also will allow us to answer the questions characterizing the vaccine strains also for the stability of the viral stock, and to allow us to supersede the existing technology.

This is a slide comparing mutation vaccine strains using the next generation technology. In this slide you can see that we were able to go through the turnover and generate more sequence data using the virus sequencing technology in this application, then the Sanger sequencing, which is considered currently to be the gold standard.

Also, this particular technology allows us to determine the low level differences which were also present in the strains of vaccinia which allow us to detect the subpopulations present within the strains. You cannot see on this slide, but you will have to take my word for it that those linked mutations -- and it is very important for the advantage of the long read of the GS flex platform that allows us to have a long read comparable to the Sanger platform, which will allow for the detection of the subpopulations with the linked mutations. In this case we saw up to ten percent of the species differentiation within the same strain.

So we are looking into that technology. We believe that just as my colleagues were pointing, generation of the data is not enough. Whatever is going into the sequencing read into the machine is absolutely crucial. We can create a bias, either under representing certain species or over representing certain species, and to draw conclusions from that can again be detrimental.

Also, as was already pointed, the different data analysis tools in the bioinformatics expertise can drastically impact the conclusions which we can draw from our metagenomics studies.

This is the data set we downloaded the day before from Delwart's group, and we analyzed it yesterday, just in a slightly different format. We can look and see that certain vaccine strains had a heavier representation of only viral sequences, and a lot of other vaccines have also a humongous amount of the bacterial DNA present in the prep. It is the same data set, just analyzed bioinformatically a different way.

It is very important to keep an open mind when looking at the data when we are evaluating the data, the data that is there. I am urging the committee to consider moving into the direction of generating of this data, at least in the initial stages of investigation. That will help the companies to have the data up front. Additional validated

techniques can be overt.

We are working with three different generation platforms. They can compensate for the deficiencies of each other. But they will allow us to generate the data, which later as the new bioinformatics techniques are developed can be revisited and reanalyzed for more meaningful results.

Thank you so much.

DR. STAPLETON: Thank you. Is there anyone else in the audience who would like to make a statement?

DR. FRANZ: My name is Dr. Holly Franz. I am from Lawrence Livermore National Lab. It was my team that worked with Eric Delwart. My team did the microarray work.

I just wanted to move on beyond that and suggest, let's not stop at microarrays or next generation sequencing, because those are available now. The cost is coming down. The PCV that several of the folks here described is very cost effective. Microarrays cost a little bit more. They don't take quite as long as a sequencing run to do, and you can get those results for about $400 for a chip. We have got chips now that do 2.1 million probes on it and can identify every virus, every bacteria, every protozoan and fungi that has been sequenced to date. We update those on a regular basis. So those are available and out there for use, or are coming that way, and I think it is advantageous to keep up on that. We have talked with some of the folks at the FDA about that.

The next generation sequencing is coming down the line. The problem is, you have got all this sequencing, you've got all this genomics, and what the hell do you do with that? The thing is, we have got a whole bunch of computer scientists, God forbid, that are now working with the biologists, that are looking at annotation of all of these proteins that the genomics is telling us.

So what are these proteins doing, what are we getting for the genomic sequencing? We need to take that to the next level of computational modeling of how these host pathogens interact with the individual host, whether it is a pig or a person or a monkey. Those will each be different, depending on the genetics of each of those individuals, and each different within the individuals within a species.

So there is a lot farther that we can go on this.

I was struck with was how retrospective all this study was on the vaccines. We have the capability now to take a look at, once a vaccine is given, what are the micro RNAs that are being produced by that individual as they are responding to that vaccine. What are the cytokine levels, what are the immunomodulators that are being released. So instead of waiting around going, hm, is that child going to get sick, are we going to have an adverse reaction, let's be a little bit more prospective and look at what is going on at the molecular level that we now have the tools to take a look

at.

Thank you.

DR. STAPLETON: Thank you. Is there anyone else that would like to make a statement?

I think the speakers and Dr. Peden raised many important issues about the rapid evolution of our ability to detect adventitious agents. I know that there have been many expression of gene arrays within individuals in human vaccines already. Those have been difficult to sort out differences, although certainly genetic polymorphisms will play a role in the way people respond to vaccines. But that has not been proven yet with use of gene expression arrays of human mRNAs.

Agenda Item: Committee Discussion

Let's go to our last discussion question and go around the table and get peoples' opinions on the last discussion point, which is, please discuss the application of emerging technologies and the implications for their use in the detection of known and unknown adventitious agents in vaccines that are currently licensed, as well as those that are under development. I think maybe I will start with Dr. Hughes, to give everyone at every end of the table to go first today.

DR. HUGHES: We are really at a marvelous point in a sense in terms of how technology is reshaping the way we

can look at problems that are both of interest to basic scientists and important for the clinic and for the licensing of vaccines and other biologicals.

It is my opinion that we already have very powerful tools; we are fortunate in that way. But I think in the near term, what is going to change is both our ability to do very broad and deep sequencing efficiently and relatively cheaply, and what will probably follow that but perhaps more slowly is our ability to mathematically or electronically analyze that data.

I think we are to the point where you can look at the horizon or maybe just the sight of the horizon, and see the power of the actual machinery that will generate the sequence. But as one of the previous speakers mentioned, that is where the problems begin.

One of the things that I think the deep sequencing technology holds out both the most promise for but also the most problems for is not in recognizing the adventitious agents or pathogens we know, but in trying to pick out the ones we don't. And of course that implies that you can find something that you don't expect to see over a background of things that you think are there. That is still going to be a hard problem.

I would suggest that if there was something we would want to try and encourage people to do, it would be to

work on more powerful ways of sifting what will be very large amounts of data which will become available to us, and particularly with trying to answer that question.

DR. STAPLETON: Thank you, Dr. Hughes. Dr. DeStefano.

DR. DESTEFANO: I guess from what I heard, it sounds like in the future we can expect ever more powerful methodologies for detecting perhaps new viruses and adventitious agents. So I can't comment on specific methodologies or technologies, et cetera.

But I think what it says to me is that we ought to start giving some thought about how we are going to start approaching and evaluating these findings that we can expect in the future.

DR. STAPLETON: I think Dr. Baylor was hoping we would have some comments on that today, which is a very difficult thing to do.

DR. TSAI: I think one aspect of the application of these new technologies that will impact industry is the validation. It is not straightforward. I'm not an expert in this area, but for individual PCVs to identify a single agent validating the assay with spiking, the various levels, characterization robustness and so forth, can take a year or in that time frame. So speaking of a procedure that would potentially encompass all known agents, perhaps in the

thousands, to attempt to validate an assay on that scale would be formidable. How to cope with that is something that companies would look forward to working with the agency to help define.

There was some mention of replacing raw materials of animal origin with recombinants. In some cases that has been done. It is not a straightforward switch. The substitution of recombinants can reduce yield significantly. So I think it is not necessarily a straightforward solution.

As GSK has mentioned, the re-derivation of cell banks and master viral seeds can essentially lead to the development of an altogether new vaccine with clinical trials and so forth. That of course has public health implications that have been discussed. There are a number of other vaccines for which there may be only one manufacturer or two, and some consideration has to be given towards risk-benefit of either removing a product or temporarily suspending its use.

I would give rabies vaccine as an example, where very recently there was a shortage and rationing of rabies vaccine because one manufacturer was temporarily off the market.

DR. ROMERO: I think we are at a very exciting time. If you look back 50 years ago with the ability to develop primary cell line culture by Enders, which opened a

tremendous window onto the identification of new viral agents, we are at that point today at a much greater magnitude, because we can now identify these new agents without having to try to culture them. This poses a lot of challenges to us as we begin to reanalyze our old methodologies and our old methods of producing vaccines.

I was struck by what Dr. Peden said. That is, poorly funded groups like our own, when he was referring to CBER's efforts. I think that if we could make a recommendation, it is to continue funding to these agencies that the FDA supports at a high level, in order so that they can embrace this. I am a believer in embracing new technologies, new methodologies, as soon as they are validated. We know that CBER has the capacity to do that.

So my statement is that we should be adopting these. We should be making the recommendations for higher levels of financial support so that they can move forward with these methodologies.

DR. GELLIN: Coming to this meeting today and then reinforced by the several infomercials, it is clear that there is a lot more of this to come. This technology is becoming more powerful and less expensive, so I think we have to anticipate that we are going to be seeing more of it.

So I think it is going to be really important that these findings, whoever finds them and how they are reported,

we have some ability to evaluate them, because they are going to come at us in a number of ways. I suspect that the findings are going to outpace their interpretation and outpace the context in which they come.

So I think we have to recognize that as we move forward. Maybe some guidance to the readers who are going to be faced with dealing with these findings, to put them in some kind of context so that they are interpretable by others than the people who are doing the work.

NASA has got a project now called the Kepler Mission, where they are looking for habitable planets. I think that as you point your telescope in different places, you are going to see more and more of these things. So we are going to see those.

In the message of, don't try this at home, we are going to see a lot of this, and we are going to see a lot of it done by people who know what they are doing and those who don't know what they are doing, which is why you don't try it at home.

I think we also should have some conversation with medical journals, who are going to be faced with a lot of potential publications about this for how they deal with this as well. We are going to see this information in many places, and all those who are going to be seeing it are going to need to be able to put it in context.

DR. STAPLETON: Thank you. In the sake of fairness, I will go next and not be last again.

I certainly agree with all that has been said. This is an exciting time. 4-5-4 sequencing and the new technologies truly are a huge advance. I take great comfort in the fact that using these methods we haven't found more in the limited application of these thus far. This JV paper, I think you looked at eight vaccines, and we didn't turn up thousands, which is a very reassuring thing to me as a virologist. I'm surprised we didn't find a lot more, honestly.

So I am encouraged by the fact that after saying that, I agree with everyone, we are going to find more. We are going to find viral sequences of unidentified viruses and viruses that we won't be able to grow, so we won't be able to tell if they are infectious in cell culture, let alone in humans. So there are some very difficult times ahead.

I think the FDA is asking us to give our thoughts on how they should develop policies. I jokingly said to a couple of people earlier that once the new technology is out and works and is validated, the cat is out of the bag. You have to use it. So I don't think there is any question that application of pyrosequencing to cell substrates that are going to go into humans is going to be the standard of the future.

I agree with Dr. Romero that FDA should have those abilities as well, so that not only do you have manufacturers evaluating this, but you have the same ability, or at least as they do now, they contract with independent laboratories, so that there is a regulatory mechanism to validate pyrosequencing data and then the follow-up validation that what you found is real.

Having said that, I don't see any way when we find these things that there is going to be a set policy that is going to address them. I think it is going to be just like this meeting, that it has got to be on a case by case basis reviewed, analyzed, and it is going to create a lot of pain, or not pain, but concern. That is the nature of advancing science and technology.

The one comment that hasn't been made about this is that this will add to the costs of producing vaccines. I think that is another issue that we have to accept to protect our children and ourselves.

So with that, I will move on to Dr. Debold.

DR. DEBOLD: I agree with much of what has been said. I guess in general I would say the technology that you have available to you right now should be used whenever possible. I am not able to comment on some of the new types of technologies that were presented here.

Prior to coming to the meeting, I did read the 2010

guidance for industry. I was surprised to find in there, requirements for testing porcine derived reagents, and it specifically mentions circovirus in there. It just seems like the material and the methods that we have now, we need to use them, and it needs to be not just a recommendation, but a requirement.

My concern is about health, and it is about public health, and it is also about public trust. We just had a new survey that came out of University of Michigan two months ago saying that 54 percent of Americans are concerned about vaccine safety now. So we have got to do whatever can be done to eliminate the use of animal products in vaccines that includes human materials.

I am also concerned about human endogenous retrovirus. We have been hearing a lot about that as it relates to MMR and its potential relationship to autoimmunity. So there is a lot of work that needs to be done.

I agree that some kind of process for dealing with the discoveries when they happen needs to be set up so that we can do a much better job of taking care of these problems.

DR. LARUSSA: Where to start? One of the things that I am concerned about is this self generating aspect of this kind of technology. I can't see us going forward in this stop-start-stop-start mode with the National Vaccine

Program. I don't see any way around using high throughput sequencing to look for other agents.

I guess what I would say is, we have to think about it in a rational way, in that we look at the agent that is identified. If there is a clear known risk associated with that, it gets acted on immediately. If it is a hypothetical or theoretical risk like the risk we are talking about today, then it is used for hypothesis generation.

The only other point that I would try to make is that this is not a substitute or a replacement for the kind of careful large vaccine safety studies that we need to do and that are going to need to continue to be done. Ultimately you need to show that a vaccine either does or does not cause a problem, and we know how to do that, and we should continue to do that.

DR. STAPLETON: Thank you. I think those are some excellent points, especially the idea that if there is biological plausibility of a risk with a new agent, that that would be a starting point for the FDA to have a marker or a signal, as opposed to a theoretical risk.

DR. GREENBERG: I agree with all the sage comments that have come before this. Most specifically it is incumbent on the scientific and medical community to try to educate the public to explain the new world that we are getting into with the depth of how deeply we can investigate

our biologic products.

That said, I do think there are some things that can be done very quickly. For example, deep sequencing has been here for what, a year and a half now, or a year. It seems to me if I were a company making vaccines, I would go to all my master seed stocks, and make sure that my cell substrates or at least know what my cell substrates have, so that you are forearmed with that, and begin to make sure that at least the raw materials with which we make vaccines -- because it is a good ten years from the time you put that in the cell substrate until the time it comes out. You don't want to start, as unfortunately happened here, with material that you could have found out was problematic from the start.

This isn't new, but I think that is what I would encourage happening very quickly.

DR. SANCHEZ: I just want to also agree with what has been said. I think these technologies are here. They will have to be incorporated into vaccine development.

I agree with you and others that it is encouraging that other agents have not been found, and also that this is just one of the many facets of vaccine, not only development but also safety and efficacy studies in the future.

DR. WHARTON: It is clear that the new technologies present an important opportunity to learn more about vaccines and how they work, and it is very exciting. They are going

to be applied to answer questions about vaccines, and it is important that that work be done very well, using validated approaches.

My scientific colleagues at FDA play a hugely important role in vaccines in the United States. I think it is important that FDA scientists have access to the expertise and capacity they need for applying these new technologies to vaccine evaluation.

That said, what this whole experience brings home to me is that with new technology, our capacity to find things greatly outstrips our capacity to understand them. So we not only need the in vivo in these new technologies and methodologies, but also in the complementary kinds of work that was described this morning, that is needed to understand what it is we find.

I agree with you that there is not going to be a rule book to follow here. It is going to be a very arduous process as things are found, and I think they will be, to understand their significance, with collection of additional information and carefully looking at things to evaluate the significance of individual findings as they occur.

DR. MC INNES: I think it was early 2005 that the first next generation sequencing machine was on the market. It has been five years already. Under the NIH human microbiome project, I have the pleasure of spending 11 hours

a week with the large scale sequencing centers, the Broad, the Baylor, JCDI and Wash U., in an attempt to identify a core microbiome in humans. The sequences are running day and night. The bioinformatics is lagging way behind in understanding what to do with the data that are being generated.

So I think we have technology. Do I think we will all go back to Sanger sequencing? No. It has changed, it has already changed. We are moving on. It is not a substitute for very careful preclinical work that is currently done. It is an addition. It is certainly not a substitute for clinical work that is done.

I am also struck by something that I hope is not going to be divisive in the community, and that it is somebody else's problem. I think we all bear a responsibility for trying to be part of the solution, not just raising the red flag about the problem. I think remaining pragmatic and optimistic is a social responsibility, in terms of protecting the health of our children and of our adults in this country and elsewhere.

So I think we won't have it go backwards, but I think it is going to be -- it is not just the identification of the adventitious agents; there is a huge amount of research that is going to have to go on to know what it means and what do we do about it. So I don't see it immediately

translating into being incorporated into requirements for testing, it may be at the identification stage, but what to do with it is a much more difficult problem.

DR. COFFIN: I am struck by a couple of things. One is, clearly technology of the 4-5-4 sequencing may not be ultimately the best way to do this. It may be there are other technologies coming along that will rapidly supplant it. But clearly this is going to become a standard for assessing these kinds of risks of adventitious agents in vaccines and other bio products.

I also can't help noting or repeating a comment that was made before. In this particular case, this could have been spotted before. It could have been spotted right away. The contamination isn't subtle. It is PCR assays, it is a known virus. Had anybody thought to look, it would have been seen right off the bat. So that also suggests that there should probably be a more careful beefing up of the way assays for known viruses are done, which would include a careful examination of all the animal products in the history of the product being used. In that case, somebody would have said, here is porcine trypsin, we are required if you like to do a scan for porcine virus.

I think a more careful attention to that issue, the low tech issue, if you like, could perhaps head off problems like the is in the future, before we get into full

implementation of ultra deep sequencing technology to do this.

That said, ultra deep sequencing technology will -- I guess you can imagine four possible outcomes. One is that you will see an agent that you know that is familiar. You know all about it, you know what to do with it, how to deal with it. Second, you will see something that is a lot like something you know or something that you know, but you didn't expect it would be there, you don't know what its properties are in this case, which is what we face today.

Third, you will see something that is clearly a virus, but one that you have never seen before, you don't know anything about it, so some substantial amount of research is needed in order to understand what it is. I suppose the fourth possibility is, some mixture of all of these things will show up.

Given all that, I think it is probably critical right now to start the process of developing appropriate standards for interpreting these kinds of data. I think it is probably going to be incumbent on FDA or somebody in industry to maybe convene some meetings or working groups to establish what can be done and can't be done, and standards for levels of contamination and bioinformatics and data processing and so on, far more than we can do in this discussion here today. I think it is important to get this

process rolling in a very real way, that way.

DR. CHEUNG: No doubt this new technology advances our understanding for at least what is present in our vaccines or any other kind of materials. This sequencing definitely gives us the extra information that is not present in the past.

Of course, as a consumer's point of view or even from the regulators' point of view or industry, we want to know what is actually there. What is actually there gives us the knowledge to decide with an informed decision to see what we want to do with the information.

So it is really important that we get that information out on the table so that we can discuss them or make decisions based on those data, factual data, not just implied or potential problems.

However, even this powerful technology is only the first step, because you only see what is there. You don't really know this agent. If it is a known agent, you have some tests to validate it, or if it is a new one, by definition there is no test, especially in terms of infectivity or pathogenesis that we covered today. So it is very important, after getting this data, that there is a lot more work to be done, decisions to be made how to use this information.

Definitely in terms of informing the public with

what is current in our vaccines, that should be made known, especially to talk about the rotaviruses; that should be made known to the parents.

So with that, the information is extremely important to be made public. Also, useful further research in other vaccine development.

Thank you.

DR. STAPLETON: Thank you. Before we open it up for open discussion, Dr. Baylor or Krause, are there any issues that you feel we have not addressed? Or are there any comments you would like to make?

DR. BAYLOR: I would like to say this has been very helpful. One of the parts of that discussion point is the implications for the use of these new technologies. I think we all agree that, whether we want to or not, these new technologies will be used. Somehow we have to get into -- I think Dr. Gilbert mentioned this in the first part of the day, about decision analysis. Maybe some comments from you to push this a little further on this decision analysis.

I believe using these technologies, we will find many unknowns, and we are going to have to be able to evaluate the unknowns, and it is going to be very difficult for the agency to convene an advisory committee every time we discover a new adventitious agent.

So I think the discussion really needs to be on

what additional components will go into a decision analysis to make those decisions across the board, so we don't have to come back to the committee every time this issue comes up.

DR. STAPLETON: Would anyone on the committee like to start? I think Dr. LaRussa's comment struck a chord with me. A set of criteria for unknown agents that are discovered, if there is any way to look at a cell type that has been used in other vaccines and their safety data, then that is relevant information for that vaccine that you are studying.

I think a large number of vaccines are produced in relatively well studied cell lines, and those cell lines, if they haven't been screened by deep sequencing yet, they probably will be in the next week.

So I think for vaccine production cell lines, you will have a background of data that you can use. If there are extensive safety data and a new adventitious agent is discovered in one vaccine, you can look for other vaccines in that very quickly. If there are extensive safety data, that would give you a lot of leeway in saying we don't have to stop. We need to study those, but we don't have to stop those vaccines today.

On the other hand, with a new cell type, I think it is going to become imperative to test that before it goes into humans. Probably an alternative cell line is going to

be the easiest step, but I will ask for other comments.

DR. LARUSSA: I think unfortunately you may not have to have a new meeting every time, but you certainly are going to have to have a lot of conference calls.

One of the benefits of doing it that way is, there is going to be a tremendous amount of information up front, and the cross fertilization that goes on with throwing an idea out among people with lots of different areas of expertise is not only going to help you with your immediate problem, do I stop use today or do I continue, but also the idea of hypothesis generation about what you want to look at in the future.

So I have no desire to come down to Gaithersburg every month to discuss these issues, but I think you are going to have to have some sort of regular way of going over these, at least until you get your feet on the ground and do enough of them so that you can establish a pattern.

DR. DEBOLD: Whatever you do, I hope that you will find a way to have sufficient public inputs into these issues. These are very big issues, and the risks are being borne by individual people, and the public has a right to know whatever it is that you learn. So I just hope that you all don't lose sight of the need to include the public.

DR. STAPLETON: That is clearly very important. The risk if you get a problem from a vaccine for that

individual is 100 percent. Similarly if you get the disease that the vaccine would have prevented it is 100 percent. So it is a difficult issue and very personal on both sides of the things.

DR. DEBOLD: I would say too, if this is going to come up a lot, you may want to look at talking to an ethicist, seeing ethically how some of this needs to be handled. This is a very complex issue that involves the health of the population as well as individuals.

Whatever process you develop, I am very pleased at the transparency here today. That needs to continue. We have to find a way to have a courageous conversation about this. I am quite certain, like everybody said, we are going to find things that maybe we should have known were there. Like on this list, porcine circovirus was clearly already on the list. What about the new things that we don't know about yet? What about the prions? What about lots of other things that I am sure we are going to find? We have to find some way of having truly informed consent about this.

DR. STAPLETON: Again, I want to thank FDA and GSK for the transparency of this process. It has been really excellent. Other comments?

Would you like to restate your question, or do you think we gave you some feedback that will be helpful there?

DR. BAYLOR: No, I think this has been very

helpful. I think those last few comments have been helpful as well.

Lastly, I would like to say that I think everybody recognizes the difficulty of this issue. Both companies have been moving very fast. Both Merck and GSK have been working very closely with the agency to try to continue this investigation and get to the root cause of this and to resolve this. So I just wanted to make sure for the record that both companies have been really forthcoming and working very closely with the agency.

DR. STAPLETON: Are there any other comments from the committee? I think Christine is amazed that we are done before 4:30. An hour ago she wouldn't have bet on that.

I think if there are no more comments, the meeting will adjourn. Thank you.

(Whereupon, the meeting was adjourned.)